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FILE 'HOME' ENTERED AT 13:51:22 ON 22 MAR 2005

=> file .biotech caplus  
COST IN U.S. DOLLARS

	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	0.21	0.21

FILES 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS'  
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## 7 FILES IN THE FILE LIST

=> s oligo-dt and VN###  
L1 13 OLIGO-DT AND VN###

```
=> dup rem l1
PROCESSING COMPLETED FOR L1
L2          7 DUP REM L1 (6 DUPLICATES REMOVED)
```

=> d ibib abs 12 1-7

L2 ANSWER 1 OF 7 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN  
ACCESSION NUMBER: 2003-23088 BIOTECHDS  
TITLE: Comparing gene expression profiles of two or more samples, by  
using sample-specific primers for cDNA synthesis and  
amplification of the synthesized cDNAs, and comparing levels  
of abundance of genes between samples;  
expression profiling and DNA primer useful for drug  
screening and diagnosis

AUTHOR: SLEPNEV V I

PATENT ASSIGNEE: SENTION

PATENT INFO: US 2003077611 24 Apr 2003

APPLICATION INFO: US 2002-113034 1 Apr 2002

PRIORITY INFO: US 2002-113034 1 Apr 2002; US 2001-346140 24 Oct 2001

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPT: 2003-625538 [59]

AN 2003-23088 BIOTECHDS

**AB DERWENT ABSTRACT:**

**NOVELTY** - Comparing gene expression profiles of two or more samples, comprising synthesizing several first strand cDNAs from first sample (S1) using oligonucleotide primer having sample-specific sequence tag, selectively amplifying subset of cDNA, detecting abundance of amplified products, where abundance determines expression profile (EP) of genes in S1, and comparing EP of genes in S1 with EP of genes in second sample, is new.

DETAILED DESCRIPTION - Comparing (M1) gene expression profiles of two or more samples, comprises: (a) synthesizing a number of first strand cDNAs from a first sample using a first oligonucleotide primer comprising a sample-specific sequence tag, where the sample-specific sequence tag is GC rich at its 5' terminal and AT rich at its 3' terminal; (b)

selectively amplifying at least a subset of the cDNA so as to generate one or more sample specific amplified products; (c) detecting the abundance of one or more sample specific amplified products, where the abundance determines an expression profile of one or more genes in the first sample; and (d) comparing the expression profile of the genes in the first sample with an expression profile of genes in a second sample, where a difference in the expression profile indicates differential expression of the genes in the two samples. INDEPENDENT CLAIMS are also included for: (1) identifying (M2) a modulator which regulates one or more gene expression in a sample, by performing M1, where the expression profile of one or more genes in S1 is compared before contacting with the modulator, with an expression profile of one or more genes in S1 after contacting the modulator, where a difference in the expression profile indicates that the modulator regulates gene expression in the sample; (2) a composition (I) for detecting the level of gene expression, comprises a first oligonucleotide primer comprising a sample-specific sequence tag, where the sample-specific sequence tag is GC rich at its 5' terminal and AT rich at its 3' terminal; and (3) a kit (II) for detecting the level of gene expression, comprises a first oligonucleotide primer comprising a sample-specific sequence tag, where the sample-specific sequence tag is GC rich at its 5' terminal and AT rich at its 3' terminal, and packaging material.

WIDER DISCLOSURE - Disclosed is a business method to conduct a pharmaceutical business.

BIOTECHNOLOGY - Preferred Method: In M1, the first oligonucleotide primer comprises at least one degenerate nucleotide. The sample-specific sequence tag comprises at least one artificial nucleotide. Synthesizing the first strand cDNAs comprises reverse transcribing RNA from two or more sample sources into first strand cDNA, where the cDNA is differentially tagged according to the sources. The first strand cDNAs are synthesized by reverse transcription using total RNAs or mRNAs derived from the first sample. A third oligonucleotide primer comprising the sequence-specific sequence tag of the first oligonucleotide primer is used for the amplifying so as to generate one or more sample-specific amplified products. The sample-specific sequence in the first oligonucleotide primer is 15-30 nucleotides, preferably 20-24 nucleotides in length. The first oligonucleotide primer further comprises a sequence of 5' **oligo(dT)n VN 3'**, where n is at least 5, V is dATP, dGTP or dCTP, and N is dTTP (or dUTP), dATP, dGTP or dCTP. The first oligonucleotide primer is provided as a mixture of primers comprising (5'-(specific sequence tag)20-24T12-16AN-3', 5'-(specific sequence tag)20-24T12-16CN-3', and 5'-(specific sequence tag)20-24T12-16GN-3'), where the specific sequence tags are identical or different for each primer in the mixture. The first oligonucleotide primer and the sample-specific sequence tag is located at the 5' of **oligo(dT)nVN**. M1 further comprising synthesizing one or more second strand cDNAs complementary to the first strand cDNAs using a second oligonucleotide primer comprising a first arbitrary sequence tag, and amplifying at least a subset of the second strand cDNAs so as to generate one or more sample specific amplified products. The second oligonucleotide primer further comprises a second sequence which is complementary to a subset of the first strand cDNAs so as to permit the synthesis of one or more second strand cDNAs. The second oligonucleotide primer is located 3' of the first arbitrary sequence. The second oligonucleotide further comprises a sequence of (Z)<sup>m</sup> between the first and second sequences, where Z is a nucleotide which can form base pair with any of A, T, G or C, and m is at least 2, preferably m is 4. The second sequence is 5-10, preferably 6-7 nucleotides in length. The arbitrary sequence within the second oligonucleotide primer comprises a A-T rich region and a G-C rich region which is located at 5' of the A-T rich region. The second oligonucleotide primer used is the same for the two or more samples to be compared. The amplification is performed by PCR. The amplifying further comprises using a fourth oligonucleotide primer which comprises the first arbitrary sequence tag of the second

oligonucleotide primer. The fourth oligonucleotide primer used is the same for the two or more samples to be compared. The second sequence within the second oligonucleotide prime is gene-family-specific, a peptide specific for a protein family, or a sequence encoding a signature sequence motif for a specific protein family. The protein family is selected from receptor tyrosine kinases, G protein coupled receptors, seven transmembrane receptors, ion channels, cytokine receptors, tumor markers, mitogen associated protein kinase (MAPK) cascade kinases, transcriptional factors, GTPases, ATPases, and development protein markers. The first strand cDNA is synthesized in a solution without attaching to a solid support, or by attaching to a solid support. The solid support is a microparticle or an inner wall of a reaction tube. M1 further comprises separating the second strand cDNA from the first strand cDNA before amplifying the second strand cDNAs. The third oligonucleotide primer is linked to a detectable label (selected from fluorescent labels, radioactive labels, colorimetric labels, magnetic labels, and enzymatic labels) or is labeled with a sample-specific label. The amplified products are sampled at a predetermined time or cycle interval during the amplification. The abundance is detected for each sampled amplified product. M1 further comprises separating the amplified products before detecting the abundance of the amplified products. The amplified products are separated and their abundance is detected by chromatography, mass spectrometry, measurement of fluorescence, or measurement of optical density. The amplified products are separated by electrophoresis, preferably capillary electrophoresis. The difference in the expression profile of the genes is measured by a ratio of sample-specific detectable labels on amplified products from the genes between two or more samples. M1 further comprises generating an amplification plot, calculating a Ct of amplification for each of the genes, and measuring the difference in the expression profile by a ratio of the Cts. M1 further comprises collecting one or more genes which are differentially expressed and identifying the sequence identities of the genes by DNA sequencing.

USE - M1 is useful for comparing gene expression profiles of two or more samples chosen from normal sample, disease sample, sample at a given development stage or condition, sample prior to a given treatment stage or condition, and a sample at a given culturing stage or condition. The samples are derived from an animal, an organ, tissue type and a cell type. M2 is useful for identifying a modulator which regulates one or more gene expressions in a sample. (I) or (II) is useful for detecting the level of gene expressions (all claimed). M1 is useful for studying normal developmental processes, for identifying potential drug targets for further research and development and useful diagnosis marker for certain pathological conditions, to study the effects of certain treatments on cells, tissues or individual, or for characterization of cells or organisms that underwent genetic modification.

EXAMPLE - RNA was prepared. Tissues were homogenized in a homogenizer at 1 ml of TRIZOL reagent per 50-100 mg of tissue of 30 seconds, followed by a final homogenization of 1 minute. The homogenized tissues were left for at least 15 minutes up to an hour, at room temperature, or they were stored in -70degreesC until needed. 0.2 ml of chloroform per ml of Trizol was added and mixed by shaking. The mixture was incubated at room temperature for 5 minutes, then centrifuged for 5 minutes. The upper phase was collected into a separate tube. 0.5 ml of isopropyl alcohol per ml of Trizol was added to precipitate RNA. The mixture was put on ice for 5 minutes and was centrifuged for 10 minutes. The supernatant was removed and the pellet was washed with 1 ml 75% EtOH per ml of Trizol, mixed, and centrifuged for 5 minutes. The supernatant was moved and the pellet was air dried for 30 minutes to 1 hour. After pellet was air dried, the pellet was resuspended in RNase free water to a desired concentration. The RNA extracted was cleaned up by adding the appropriate volume of buffer RLT, and mixed thoroughly. An appropriate volume of ethanol (96-100%) was added to the diluted RNA and mixed thoroughly by shaking vigorously. The sample was applied to an RNeasy midi spin column or RNeasy maxi spin column and was placed in a 15 ml or

50 ml centrifuge tube and centrifuged. The flow-through was discarded. Reverse transcription in solution was performed. The RNA samples (1-5 μl) were mixed with 1 μl of dNTPs solution (10 mM) and 0.0005-0.5 μM (final concentration in 20 μl mixture) of first oligonucleotide, heated for 7 minutes at 70°C, and cooled for 2 minutes at 4°C. The above mixture was then mixed with the reaction mixture (4 μl reverse transcriptase (RT) buffer (250 mM Tris-HCl) (pH 8.3 at 25°C), 375 mM KCl, 15 mM MgCl<sub>2</sub>, 2 μl 0.1 M dithiothreitol (DTT), 1 μl RNase inhibition and 1 μl of SuperScriptII reverse transcriptase and 5-10 μl of water) in a total volume of 20 μl. The reverse transcription reaction was incubated for 1-2 hours at 45°C and was terminated by heating at 65°C for 10 minutes. An aliquot of sample (5-20 μl) was directly analyzed by PCR. Optionally, the RNA templates were degraded by incubation with RNase H enzyme prior to PCR amplification. Second strand synthesis was performed. The synthesis of the second strand of bound DNA was performed with mixture of Taq polymerase (0.5-1.5 microns) and Pwo DNA polymerase in PCR thermocycler. The reaction mixture included 6-10 μl of cDNA on oligobeads from RT reaction, 5 μl of 10xPCR buffer or 10xRT-PCR buffer (500 mM Tris, 200 mM KCl, 100 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 2.5 mM Mg<sub>2</sub>Cl<sub>2</sub>, pH 8.5), 0.1 mM dNTPs, 0.5-1 μl of second primer (100 μM) in a total volume of 50 μl. The synthesized second DNA strand was removed from the beads at 96°C and used for further amplification. PCR amplification of synthesized cDNA (5-20 μl) was performed in the presence of 10 μl of 10xPCR buffer or 10xRT-PCR buffer, 2-3 mM MgCl<sub>2</sub>, 0.05-0.2 mM dNTPs, 0.1-1 μM of third primers labeled with either FAM, Rox, or Hex, 0-1 μM of unlabeled third primers, 1-2 μl of fourth primer, 0-5% dimethylsulfoxide (DMSO), 0.5-2 microns of proofreading DNA polymerase (Pwo or Tgo) and 1.5-3 microns of hot-start DNA polymerase. The amplification was conducted using I-cycler or PCR Express. Aliquots of samples (typically 25 μl) were withdrawn after each cycle at the end of extension step (72°C) starting with 10-15th cycle. Equal volume of PCR mix containing primers, polymerase and dNTPs was placed into reaction mix after each sample removal. The collected samples were analyzed. Capillary electrophoresis was performed on SCE 9610 fully automated 96-capillary electrophoresis genetic analysis system. (40 pages)

L2 ANSWER 2 OF 7 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN

ACCESSION NUMBER: 2002-17928 BIOTECHDS

TITLE: Identifying and characterizing gene expression in samples, for identifying mRNAs expressed at different levels, comprises employing an identimer having a **oligo-dT** primer of a specific sequence and a detectable marker at its 5' end; gene expression identification and characterization, database and computer bioinformatic software

AUTHOR: KANE M D; DOMBKOWSKI A A; NAGEL A C

PATENT ASSIGNEE: GENOMIC SOLUTIONS INC

PATENT INFO: WO 2002036828 10 May 2002

APPLICATION INFO: WO 2000-US45401 1 Nov 2000

PRIORITY INFO: US 2000-244933 1 Nov 2000

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2002-508123 [54]

AN 2002-17928 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - Systems for identification and characterization of gene expression in one or more samples, comprise an identimer having a specific **oligo-dT** primer sequence, where the identimer comprises a detectable marker at its 5' end.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a system (M1) for identification and characterization of gene expression in one or more samples, comprising: (a) providing one or more samples comprising one or more mRNA molecules; (b) providing an

identimer comprising an **oligo-dT** primer of sequence, from 5' to 3' end, of (I) or (II), where the identimer also comprises a detectable marker at its 5' end; (c) contacting the mRNA with the identimer so that the polyT portion of the identimer hybridizes to the polyA tail of the mRNA and the **VNx** portion of the identimer hybridizes with portions of the mRNA immediately upstream of the polyA tail; (d) reverse transcribing the mRNA to produce a first strand cDNA that includes the identimer; (e) synthesizing a second DNA strand complementary to the first strand cDNA to form a duplex; (f) cleaving the duplex with a sequence-specific cleaving agent to provide one or more duplex cleavage fragments; (g) ligating an adaptamer comprising an RNA polymerase promoter site to one or more of the cleavage fragments; and (h) amplifying one or more ligated cleavage fragments using the identimer to produce one or more amplified fragments comprising sequences complementary to a 3' end of the mRNA; (2) a system for identification and characterization of gene expression in one or more samples, by: (a) employing steps (a) - (c) of M1; (b) providing a second sample comprising one or more mRNA molecules; (c) providing an identimer comprising (I) or (II); and (d) employing steps (c)-(h) of M1; (3) a system for identification and characterization of gene expression in one or more samples, by employing the steps of M1, and further contacting the in vitro transcribed RNA with the identimer so that the polyT portion of the identimer hybridizes to the polyA tail of the in vitro transcribed RNA and the (I) or (II) portion of the identimer hybridizes with portions of the in vitro transcribed RNA immediately upstream of the polyA tail, and reverse transcribing the in vitro transcribed RNA to produce a first strand cDNA that includes the identimer; (4) a system for identification and characterization of gene expression in two or more samples, comprising: (a) employing steps (a) - (c) of M1; (b) providing a second sample comprising one or more mRNA molecules; (c) providing an identimer comprising (I) or (II); (d) employing steps (d) - (h) of M1; (e) contacting the in vitro transcribed RNA with the identimer so that the polyT portion of the identimer hybridizes to the polyA tail of the in vitro transcribed RNA and the (I) or (II) portion of the identimer hybridizes with portions of the in vitro transcribed RNA immediately upstream of the polyA tail; and (f) reverse transcribing the in vitro transcribed RNA to produce a first strand cDNA that includes the identimer; (5) a kit comprising: (a) one or more identimers comprising an **oligo-dT** primer of sequence, from 5' to 3' end, of (I), where the identimer also comprises a detectable marker at its 5' end; and (b) one or more sequence-specific cleaving agents. Tn-**VN<sub>n</sub>** (I)  
Tn-**VNNN** (II) n = an integer 8 or greater but not more than 50 representing the number of T's; V = a nucleotide a, c, or g but not t; N = a nucleotide a, c, g, or t; and x = an integer 3 or greater but not more than 10 representing the number of N nucleotides.

**BIOTECHNOLOGY - Preferred Method:** The system further comprises identifying and characterizing the cleavage fragments according to the presence of the marker, the sequences corresponding to the (I) or (II) nucleotide sequence and the sequence associated with the sequence-specific cleaving agent, and the size of the fragment. The system also includes identifying any gene associated with the cleavage fragments by comparing the sequence and size characteristics of the cleavage fragment with a database contacting sequence and size characteristics of RNAs associated with known genes, where the comparison is conducted by means of software operated on a microprocessor.

**USE -** The system is useful for identifying any or all genes expressed in a given in vivo or in vitro RNA sample, as well as the relative differences in mRNA between 2 or more samples, where desired, for supporting discovery of new genes, and for identifying mRNAs that are expressed at different levels between 2 or more samples.

**ADVANTAGE -** The new system or method addresses limitations of prior methods by comprising compositions and systems that incorporate new strategies where molecular or biochemical assay compositions and systems are linked to DNA or RNA sequence databases for optimal resource

efficiency in assaying gene expression. The system has the following advantages over prior and existing methods: (a) prior sequence information or clone library construction is not needed to enable the assay; (b) provides immediate sequence information in addition to information concerning changes or differences in mRNA level, to determine mRNA expression level and mRNA identification in one assay; (c) generates cDNA fragments from all mRNAs present in the sample for subsequent investigation by common molecular biology techniques; and (d) does not require prior knowledge of the sequence of the genome of the organism under investigation and can be employed in organisms lacking significant genomic sequence information.

EXAMPLE - Experimental protocols are described but no results were given. (45 pages)

L2 ANSWER 3 OF 7 MEDLINE on STN DUPLICATE 1  
ACCESSION NUMBER: 2002389129 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 12137780  
TITLE: Sensitivity and accuracy of quantitative real-time polymerase chain reaction using SYBR green I depends on cDNA synthesis conditions.  
AUTHOR: Lekanne Deprez Ronald H; Fijnvandraat Arnoud C; Ruijter Jan M; Moorman Antoon F M  
CORPORATE SOURCE: Experimental and Molecular Cardiology Group, Academic Medical Center, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands.. r.h.lekanne@amc.uva.nl  
SOURCE: Analytical biochemistry, (2002 Aug 1) 307 (1) 63-9.  
Journal code: 0370535. ISSN: 0003-2697.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200302  
ENTRY DATE: Entered STN: 20020725  
Last Updated on STN: 20030211  
Entered Medline: 20030210

AB The recent development of real-time PCR has offered the opportunity of sensitive and accurate quantification of mRNA levels that is crucial in biomedical research. Although reverse transcription (RT)-PCR is at present the most sensitive method available, many low abundant mRNAs are, although detectable, often not quantifiable. Here we report an improved two-step real-time RT-PCR procedure using SYBR green I and the LightCycler that better permits accurate quantification of mRNAs. Omission of dithiothreitol from the cDNA synthesis reaction was found to be crucial. This resulted in a lower cycle number at which the cDNA level is determined (C(T) value), steeper amplification curves, and removal of background fluorescence in the subsequent PCR. In addition, the choice of the cDNA priming oligo can improve detection sensitivity even further. In contrast to hexamer primer usage, both gene-specific and **oligo-dT(VN)** priming were very efficient and accurate, with gene-specific priming being the most sensitive. Finally, accurate quantification of mRNAs by real-time PCR using SYBR green I requires verification of the specificity of PCR by both melting curve and gel analysis.

L2 ANSWER 4 OF 7 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN  
ACCESSION NUMBER: 2001:519205 BIOSIS  
DOCUMENT NUMBER: PREV200100519205  
TITLE: Comparison of probe labeling methods for cDNA microarray studies.  
AUTHOR(S): Modrusan, Zora [Reprint author]; Young, Laura S. [Reprint author]; Cisneros, Yvette G. [Reprint author]; Reynolds, Mark A. [Reprint author]  
CORPORATE SOURCE: Microarray Systems, Incyte Genomics, Fremont, CA, USA  
SOURCE: International Genome Sequencing and Analysis Conference,

(2000) Vol. 12, pp. 80. print.  
Meeting Info.: 12th International Genome Sequencing and Analysis Conference. Miami Beach, Florida, USA. September 12-15, 2000.

DOCUMENT TYPE: Conference; (Meeting)  
Conference; Abstract; (Meeting Abstract)  
Conference; (Meeting Poster)

LANGUAGE: English

ENTRY DATE: Entered STN: 7 Nov 2001

Last Updated on STN: 23 Feb 2002

AB DNA microarrays have been a vital tool in populating gene expression databases. Competitive hybridization between two differentially labeled probe populations was used to obtain such information on Incyte's Gene Expression Microarrays (GEMs). cDNA probes were generated from 200 ng mRNA using fluorescently labeled random nonamers (GEMBrightTM). Probe profiling on PAGE and fluorescence quantitation was used to determine the efficiencies of Cy3 and Cy5 dye incorporation. These methods were developed, in part, to prevent the use of failed probe reactions. Performance of GEMBrightTM probes was compared to a commonly used DNA microarray probe labeling method, **oligo dT** priming. Probes generated with dT17 and dT17 (**VN**) primers were characterized similar to GEMBrightTM probes. Following GEM hybridizations, a non-specific signal mediated by yeast control fragments was observed in the presence of standard blocking reagents. Similar results were also observed with another **oligo dT** priming method, the 3DNATM Expression Array Detection Kit from Genisphere. This study shows performance characteristics of the GEMBrightTM labeling system and demonstrates that a careful consideration of probe labeling methods is essential for populating gene expression databases.

L2 ANSWER 5 OF 7 MEDLINE on STN DUPLICATE 2  
ACCESSION NUMBER: 1999141232 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 9973624  
TITLE: Increased specificity of reverse transcription priming by trehalose and oligo-blockers allows high-efficiency window separation of mRNA display.  
AUTHOR: Mizuno Y; Carninci P; Okazaki Y; Tateno M; Kawai J; Amanuma H; Muramatsu M; Hayashizaki Y  
CORPORATE SOURCE: Laboratory for Genome Exploration Research Project, Genomic Sciences Center (GSC), Institute of Physical and Chemical Research (RIKEN), Koyadai 3-1-1, Tsukuba, Ibaraki 305-0074, Japan.  
SOURCE: Nucleic acids research, (1999 Mar 1) 27 (5) 1345-9.  
Journal code: 0411011. ISSN: 0305-1048.  
PUB. COUNTRY: ENGLAND: United Kingdom  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199904  
ENTRY DATE: Entered STN: 19990504  
Last Updated on STN: 19990504  
Entered Medline: 19990422

AB We have developed a method for high-efficiency window separation of cDNA display by increasing the specificity of priming in reverse transcription. In the conventional method, two-base anchored **oligo(dT)** primers (5'dT16VN3', where N is any base and V is G, A or C) are used to make windows for the display of transcripts. However, reverse transcriptase often extends misprimed oligonucleotides. To avoid mispriming from dT16VN primers, we have developed two new technologies. One is higher temperature priming with reverse transcriptase thermoactivated by the disaccharide trehalose. The other is the use of competitive oligonucleotide blockers that hybridize to the non-selectively primed mRNAs, preventing the mispriming from the **VN** site. These methods were combined to improve restriction landmark cDNA scanning

(RLCS), resulting in the elimination of the redundant signals that appear in different windows. This was achieved by the increased specificity of initiation of reverse trans-cription from the beginning of poly(A) sites. This method paves the way for the precise visualization of transcripts to allow expression profiles in individual tissues and at each developmental stage to be understood.

L2 ANSWER 6 OF 7 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 97:113853 SCISEARCH

THE GENUINE ARTICLE: WE924

TITLE: DD/AP-PCR: Combination of differential display and arbitrarily primed PCR of **oligo(dT)** cDNA

AUTHOR: Rothschild C B (Reprint); Brewer C S; Bowden D W

CORPORATE SOURCE: WAKE FOREST UNIV, BOWMAN GRAY SCH MED, DEPT BIOCHEM, WINSTON SALEM, NC 27157 (Reprint)

COUNTRY OF AUTHOR: USA

SOURCE: ANALYTICAL BIOCHEMISTRY, (1 FEB 1997) Vol. 245, No. 1, pp. 48-54.

Publisher: ACADEMIC PRESS INC JNL-COMP SUBSCRIPTIONS, 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495.

ISSN: 0003-2697.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE

LANGUAGE: English

REFERENCE COUNT: 11

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB In this report we describe the first direct comparison of differential display (DD) and arbitrarily primed PCR (AP-PGR) amplification of **oligo(dT)**-primed cDNA. Our results indicate that both of these widely used RNA fingerprinting techniques have their respective advantages and limitations. DD produces profiles specific to the anchored **oligo(dT)** primer used for cDNA synthesis. AP-PCR displays significant redundancy of profiles generated from different **oligo(dT)** cDNA pools, but is not as biased to the isolation of AT-rich or 3' sequences. It was found that both techniques can utilize cDNA synthesized using a generic anchored **oligo(dT)** primer (dT(12)**VN**; equimolar amounts of dT(12)**VA**, dT(12)**VC**, dT(12)**VG**, and dT(12)**VT**, where V is dA, dC, or dG); this efficiently selects for poly(A) (+) sequences from total RNA, and significantly reduces the number of cDNA preparations required per experiment. Using dT(12)**VN** cDNA pools generated from rat liver, spleen, and brain, the two approaches (AP-PCR and DD) were used in combination. Several known mRNAs were identified; some were unique to either technique and some were common to both. Since it is the RNA which is usually the limiting resource, maximum utilization may be achieved by generating a single pool of dT(12)**VN**-primed cDNA and performing both AP-PCR and DD (DD/AP-PCR). (C) 1997 Academic Press.

L2 ANSWER 7 OF 7 CAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1995:52095 CAPLUS

DOCUMENT NUMBER: 122:232159

TITLE: Isolation and nucleotide sequence analysis of a partial cDNA clone for potato virus Y-**VN** (Korean isolate) genome

AUTHOR(S): Cho, Hye Sun; Jun, Se Il; Kwon, Seok Yoon; Park, Eun Kyung; Paek, Kee Yoeup; Paek, Kyung-Hee

CORPORATE SOURCE: Genetic Eng. Res. Inst., KIST, Taejon, 305-600, S. Korea

SOURCE: Molecules and Cells (1994), 4(2), 143-8

DOCUMENT TYPE: Journal

LANGUAGE: English

CODEN: MOCEEK; ISSN: 1016-8478

AB Potato virus Y-VN (PVY-VN) isolated from Nicotiana tabacum var. "Burley 21" in Korea was verified by electron microscopy observations of purified virions and SDS-PAGE anal. of viral proteins. RNA was isolated from the purified PVY particles and about 10 µg of RNA was obtained from approx. 500 µg of particles. Double strand cDNA was synthesized by using oligo(dT) primer and cloned in a SmaI site of pUC19. The size of cDNA inserts was estimated by restriction enzyme digestion analyses. The identification of coat protein gene of PVY-VN was done by Southern blot anal. using a radiolabeled 900 bp fragment of PVY (isolate Amigo) as a probe. The insert of the longest cDNA (clone 18) was subcloned into pUC19 and subjected to sequencing anal. The nucleotide sequences of clone 18 reported here comprise the entire viral coat protein gene and almost the full length of the nuclear inclusion protein b (NIb) gene region, totalling 2,541 nucleotides in length. The PVY-VN coat protein (CP) starts at G1,408 and codes for 267 amino acids with Mr = 30,062. At the amino acid level the CP sequence of PVY-VN strain is 93 to 96% homologous with those PVY strains whose CP sequences are known.

=> differential display and VN###

DIFFERENTIAL IS NOT A RECOGNIZED COMMAND

The previous command name entered was not recognized by the system.

For a list of commands available to you in the current file, enter "HELP COMMANDS" at an arrow prompt (>).

=> s differential display and VN##

L3 20 DIFFERENTIAL DISPLAY AND VN##

=> dup rem 13

PROCESSING COMPLETED FOR L3

L4 8 DUP REM L3 (12 DUPLICATES REMOVED)

=> d ibib abs 14 1-8

L4 ANSWER 1 OF 8	MEDLINE on STN	DUPLICATE 1
ACCESSION NUMBER:	2004124362 MEDLINE	
DOCUMENT NUMBER:	PubMed ID: 15015574	
TITLE:	In-silico analysis of kallikrein gene expression in pancreatic and colon cancers.	
AUTHOR:	Yousef George M; Borgono Carla A; Popalis Cynthia; Yacoub George M; Polymeris Mary-Ellen; Soosaipillai Antoninus; Diamandis Eleftherios P	
CORPORATE SOURCE:	Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, Ontario, Canada.	
SOURCE:	Anticancer research, (2004 Jan-Feb) 24 (1) 43-51. Journal code: 8102988. ISSN: 0250-7005.	
PUB. COUNTRY:	Greece	
DOCUMENT TYPE:	Journal; Article; (JOURNAL ARTICLE)	
LANGUAGE:	English	
FILE SEGMENT:	Priority Journals	
ENTRY MONTH:	200405	
ENTRY DATE:	Entered STN: 20040313 Last Updated on STN: 20040522 Entered Medline: 20040521	

AB Human kallikreins are a cluster of 15 serine protease genes located in the chromosomal band 19q13.4, a non-randomly rearranged region in many solid tumors, including pancreatic cancer. We utilized the SAGE and EST databases of the Cancer Genome Anatomy Project to perform in-silico analysis of kallikrein gene expression in normal and cancerous pancreatic and colon tissues and cell lines using virtual Northern blotting (VN), digital differential display (DDD) and X-profiler. At least two kallikreins, KLK6 and KLK10, are significantly up-regulated in pancreatic cancer. We probed 2 normal and 6 pancreatic

cancer SAGE libraries with gene-specific tags for each of these kallikreins. KLK6 was found to be expressed in 5/6 cancer libraries and showed the most marked (5-fold) increase in average expression levels in cancer vs. normal. These data were verified by screening the EST databases, where all mRNA clones isolated were from cancerous libraries, with no clones detected in normal pancreatic tissues or cell lines. X-profiler comparison of two pools of normal and cancerous pancreatic libraries further verified the significant increase of KLK6 expression levels in pancreatic cancer. DDD data showed a 13-fold increase in KLK10 expression in pancreatic cancer. Three kallikrein genes, KLK6, 8 and 10 are overexpressed in colon cancer compared to normal colon, while one kallikrein, KLK1, is down-regulated. While no expression of KLK6 was detected in normal colon, KLK6-specific tags were detectable in 2 cancer libraries. Similar results were obtained by EST screening; no KLK6 clones were detected in any of the 28 normal libraries examined, while 10 KLK6 EST clones were found in colon adenocarcinoma. KLK10 was not detectable in normal colon. Gene-specific tags were, however, detectable with high density in colon cancer and 7 EST clones were found to be expressed in colon Adenocarcinoma.

L4 ANSWER 2 OF 8 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN  
ACCESSION NUMBER: 2004-01800 BIOTECHDS

TITLE: Identifying target epitope or antigen comprises measuring cross reactivity of cytotoxic T cells specific for gene product differentially expressed by a cell which induces cytotoxic T cells;  
disease specific target epitope identification and virus vector for use in disease therapy and gene therapy

AUTHOR: ZAUDERER M

PATENT ASSIGNEE: ZAUDERER M

PATENT INFO: US 2003133917 17 Jul 2003

APPLICATION INFO: US 1997-935377 22 Sep 1997

PRIORITY INFO: US 1997-935377 22 Sep 1997; US 1997-935377 22 Sep 1997

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2003-829629 [77]

AN 2004-01800 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - Identifying (M1) a target epitope or antigen, comprising providing cytotoxic T cells specific for gene product differentially expressed by cell which express the target epitope, and measuring cross reactivity of the cytotoxic T cells for the cell in which target epitopes are identified as gene product which induces cytotoxic T cells, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a viral vector (I) containing a DNA insert flanked by unique sites for restriction enzymes positioned so that religation orientation of the insert DNA is fixed and the DNA insert is operatively associated with a strong regulatory element; and (2) a vaccinia viral vector (II) containing a DNA insert flanked by unique sites for restriction enzymes positioned so that religation of the viral vectors arms is prevented and the orientation of the insert DNA is fixed and the DNA insert is operatively associated with a strong regulatory element where the DNA insert encodes a target epitope identified by (M1) or a tumor specific target epitope.

BIOTECHNOLOGY - Preferred Method: In (M1), a modified differential display method is employed that increases resolution of DNA fragments and reduces the frequency of false positives. (M1) further involves the use of DNA fragments to isolate longer gene products following solution hybridization to single strand circles rescued from a phagemid DNA library. In (M1), the target epitope is specific to a cell infected with a virus, fungus, Mycobacteria or an autoimmune disease. Preferred Viral Vector: (I) is constructed by trimolecular recombination and is a vaccinia viral vector. (I) is derived by recombination with plasmid p7.5/tk or its derivatives or only

recombination with plasmid pt1/tk or its derivatives.

ACTIVITY - Cytostatic. No biological data given.

MECHANISM OF ACTION - Vaccine. No biological data given.

USE - (M1) is useful for identifying target epitope or antigen, e.g., tumor specific target epitope or antigen which involves providing cytotoxic T cells specific for a gene product differentially expressed by a tumor cell which express the target epitope and measuring cross reactivity of the cytotoxic T cells for the tumor cell in which target epitopes are identified as the gene product which induces cytotoxic T cells. The tumor cell is derived from a single immortalized, non-tumorigenic cell line. The assay is performed on a panel of tumor cell lines each derived independently from a single non-tumorigenic cell. The generated cytotoxic T cells which react to tumor cells do not react to non-tumorigenic T cells (all claimed). A vaccinia viral vector (II) is useful as a vaccine for preventing or treating tumors in mammals, including humans. (M1) is also useful for identifying target antigens in other target cells against which it is desirable to induce cell-mediated immunity. (II) is also used for booster immunization subsequent to primary immunization.

ADMINISTRATION - (II) is administered orally, intradermally, intramuscularly, intraperitoneally, intravenously, sub-cutaneously, intranasally, transdermally or rectally. No dosage given.

ADVANTAGE - (M1) identifies potential antigens expressed not only by the pathogen but also by the host cell where gene expression is altered as a result of infection.

EXAMPLE - Vaccinia virus transfer plasmid pJ/K, a pUC13 derived plasmid with a vaccinia virus thymidine kinase gene containing an in-frame NotI site were modified to incorporate a strong vaccinia virus promoter followed by NotI and ApaI restriction sites. Two different vectors, p7.5/tk and pEL/tk, comprising either the 7.5K vaccinia virus promoter or a strong synthetic early/late (E/L) promoter were produced. The ApaI site was preceded by a strong translational initiation sequence including the ATG codon. This modification was introduced within the vaccinia virus thymidine kinase (tk) gene so that it was flanked by regulatory and coding sequences of the viral tk gene. The modifications with tk gene of these two new plasmid vectors were transferred by homologous recombination in the flanking tk sequences into the genome of the Vaccinia Virus WR strain derived vNotI-vector to generate new viral vectors v7.5/tk and vEL/tk. Two large viral DNA fragments were isolated each including a separate non-homologous segment of the vaccinia tk gene and together comprising all the genes required for assembly of infectious viral particles. Recombinant vaccinia virus were generated by homologous recombination. A further significant increase in the frequency of viral recombinants was obtained by transfection of Fowl Pox Virus (FPV) infected cells with a mixture of recombinant plasmids and the two large approximately 80 kilobases and 100 kilobases fragments of vaccinia virus v7.5/tk DNA produced by digestion with NotI and ApaI restriction endonucleases. Because the NotI and ApaI sites have been introduced into the tk gene, each of these large vaccinia DNA arms includes a fragments of the tk gene. Since there is no homology between the two tk gene fragments, the only way the two vaccinia arms can be linked was by bridging through the homologous tk sequences that flank the inserts in the recombinant transfer plasmid. The results showed that greater than 99 % of infectious vaccinia virus produced in triply transfected cells was recombinant for a DNA insert. The tri-molecular recombination strategy yielded close to 100 % viral recombinants. (47 pages)

L4 ANSWER 3 OF 8 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on  
STN

ACCESSION NUMBER: 2001:873447 SCISEARCH

THE GENUINE ARTICLE: 485YP

TITLE: Detection of an mRNA polymorphism by differential display

AUTHOR: Liang S (Reprint); Rossby S P; Liang P; Shelton R C;

CORPORATE SOURCE: Manier D H; Chakrabarti A; Sulser F  
Vanderbilt Univ, Med Ctr, Dept Cell Biol, Nashville, TN  
37232 USA; Vanderbilt Univ, Med Ctr, Dept Psychiat,  
Nashville, TN 37232 USA; Vanderbilt Univ, Med Ctr, Dept  
Pharmacol, Nashville, TN 37232 USA

COUNTRY OF AUTHOR: USA

SOURCE: MOLECULAR BIOTECHNOLOGY, (OCT 2001) Vol. 19, No. 2, pp.  
121-124.

Publisher: HUMANA PRESS INC, 999 RIVERVIEW DRIVE SUITE  
208, TOTOWA, NJ 07512 USA.

ISSN: 1073-6085.

DOCUMENT TYPE: Article; Journal

LANGUAGE: English

REFERENCE COUNT: 5

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB      **Differential Display** (DD) technology was utilized to compare programs of gene expression in primary cultures of human skin fibroblasts from normal volunteers and patients diagnosed with melancholic depression. Polymorphic transcripts of a single gene differing by one tandem repeat sequence of four nucleotides (TGAT) in the 3' noncoding region were detected.

L4    ANSWER 4 OF 8      MEDLINE on STN  
ACCESSION NUMBER: 2000218682      MEDLINE  
DOCUMENT NUMBER: PubMed ID: 10753647  
TITLE: Metallothionein-null mice express altered genes during development.  
AUTHOR: Kimura T; Oguro I; Kohroki J; Takehara M; Itoh N; Nakanishi T; Tanaka K  
CORPORATE SOURCE: Department of Toxicology, Graduate School of Pharmaceutical Sciences, Osaka University, 1-6, Yamada-oka, Suita, Osaka, 565-0871, Japan.  
SOURCE: Biochemical and biophysical research communications, (2000 Apr 13) 270 (2) 458-61.  
Journal code: 0372516. ISSN: 0006-291X.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200005  
ENTRY DATE: Entered STN: 20000518  
Last Updated on STN: 20000518  
Entered Medline: 20000508

AB    Metallothionein (MT) can modulate transcriptional activity in vitro. We examined whether the absence of MT affects gene expression in vivo. We compared the hepatic RNA profiles of wild-type and MT-null neonatal mice using improved **differential display**. The hepatic MT level was maximal during neonatal development. We identified five cDNA fragments that were expressed in MT-null mice at different levels from those in wild-type mice. Two were fragments of MT-I and mutant MT-I cDNA. The sequences of the other cDNA fragments were identical to those of contrapsin, transketolase, and vanin-3. The latter two were up-regulated, whereas contrapsin was down-regulated in neonatal MT-null mice. These mRNA levels were remarkably different between the two strains of neonatal mice. Further characterization of the regulated mRNA identified here will determine whether or not they are primary or secondary effects of an MT deficiency.

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L4    ANSWER 5 OF 8      BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN  
DUPLICATE 2  
ACCESSION NUMBER: 1999:187773      BIOSIS  
DOCUMENT NUMBER: PREV199900187773  
TITLE: Increased specificity of reverse transcription priming by

AUTHOR(S): Mizuno, Yosuke; Carninci, Piero; Okazaki, Yasushi; Tateno, Minako; Kawai, Jun; Amanuma, Hiroshi; Muramatsu, Masami; Hayashizaki, Yoshihide [Reprint author]

CORPORATE SOURCE: Laboratory for Genome Exploration Research Project, Genomic Sciences Center (GSC) and Genome Science Laboratory, Institute of Physical and Chemical Research (RIKEN), Koyadai 3-1-1, Tsukuba, Ibaraki, 305-0074, Japan

SOURCE: Nucleic Acids Research, (March 1, 1999) Vol. 27, No. 5, pp. 1345-1349. print.

DOCUMENT TYPE: Article

LANGUAGE: English

ENTRY DATE: Entered STN: 5 May 1999  
Last Updated on STN: 5 May 1999

CODEN: NARHAD. ISSN: 0305-1048.

AB We have developed a method for high-efficiency window separation of cDNA display by increasing the specificity of priming in reverse transcription. In the conventional method, two-base anchored oligo(dT) primers (5'dT16VN3', where N is any base and V is G, A or C) are used to make windows for the display of transcripts. However, reverse transcriptase often extends misprimed oligonucleotides. To avoid mispriming from dT16VN primers, we have developed two new technologies. One is higher temperature priming with reverse transcriptase thermoactivated by the disaccharide trehalose. The other is the use of competitive oligonucleotide blockers that hybridize to the non-selectively primed mRNAs, preventing the mispriming from the VN site. These methods were combined to improve restriction landmark cDNA scanning (RLCS), resulting in the elimination of the redundant signals that appear in different windows. This was achieved by the increased specificity of initiation of reverse transcription from the beginning of poly(A) sites. This method paves the way for the precise visualization of transcripts to allow expression profiles in individual tissues and at each developmental stage to be understood.

L4 ANSWER 6 OF 8 MEDLINE on STN DUPLICATE 3  
ACCESSION NUMBER: 97463936 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 9322637  
TITLE: Identification of gestationally regulated genes in rat myometrium by use of messenger ribonucleic acid differential display.  
AUTHOR: Chien E K; Tokuyama Y; Rouard M; Phillippe M; Bell G I  
CORPORATE SOURCE: Department of Obstetrics and Gynecology, University of Chicago, IL 60637, USA.  
CONTRACT NUMBER: DK-20595 (NIDDK)  
FD-R-001114 (FDA)  
HD-22063 (NICHD)  
SOURCE: American journal of obstetrics and gynecology, (1997 Sep 177 (3) 645-52.  
Journal code: 0370476. ISSN: 0002-9378.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals  
ENTRY MONTH: 199710  
ENTRY DATE: Entered STN: 19971224  
Last Updated on STN: 19971224  
Entered Medline: 19971028

AB OBJECTIVE: We hypothesized that the proteins contributing to myometrial changes during gestation could be identified indirectly by analyzing the changing pattern of messenger ribonucleic acid expression in the myometrium during pregnancy. STUDY DESIGN: Ribonucleic acid was extracted from myometrium of timed pregnant Sprague-Dawley rats on days 12, 16, 20, 21, and 22 of pregnancy and on day 1 post partum. The technique of

messenger ribonucleic acid **differential display**, a simple and sensitive polymerase chain reaction-based method for rapidly identifying messenger ribonucleic acids whose levels increase or decrease, was performed with the nine different anchoring primers (oligodeoxythymidine11 VN: V = G, A, or C; N = G, A, or C) in combination with 24 different 10-base oligonucleotides of random sequence. The polymerase chain reaction products were separated by electrophoresis on a 5% polyacrylamide sequencing gel, and those whose levels changed were then cloned, sequenced, and compared with those in the GenBank database to determine whether they corresponded to a known sequence in the database or were novel. Semiquantitative reverse transcriptase-polymerase chain reaction was used to confirm differential expression of selected products.

**RESULTS:** Messenger ribonucleic acid **differential display** revealed > 500 polymerase chain reaction products that were differentially expressed during gestation, 179 of which were cloned and sequenced. Of these, 157 were from messenger ribonucleic acids whose levels increased during gestation, and 22 were from transcripts that decreased. Eighty-seven (49%) were related to sequences in the GenBank database, of which 62 (35%) were from messenger ribonucleic acids encoding known proteins and 25 (14%) corresponded to known expressed sequence tags. The technique of semiquantitative reverse transcriptase-polymerase chain reaction confirmed the increased expression of messenger ribonucleic acids encoding beta-tropomyosin, type II phosphatidyl inositol-4-phosphate 5-kinase, and a novel myometrial messenger ribonucleic acid named RPU0901AC.

**CONCLUSION:** Messenger ribonucleic acid **differential display** is a simple and sensitive method for rapidly identifying myometrial messenger ribonucleic acids that are differentially regulated during pregnancy. The identification of these differentially expressed messenger ribonucleic acids may lead to a better understanding of the molecular basis of normal and abnormal parturition.

L4 ANSWER 7 OF 8 MEDLINE on STN DUPLICATE 4  
ACCESSION NUMBER: 97411045 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 9266082  
TITLE: Automated **differential display** using a  
fluorescently labeled universal primer.  
AUTHOR: Smith N R; Aldersley M; Li A; High A S; Moynihan T P;  
Markham A F; Robinson P A  
CORPORATE SOURCE: Molecular Medicine Unit, St. James's University Hospital,  
Leeds, England, UK.  
SOURCE: BioTechniques, (1997 Aug) 23 (2) 274-9.  
Journal code: 8306785. ISSN: 0736-6205.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Report; (TECHNICAL REPORT)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199710  
ENTRY DATE: Entered STN: 19971105  
Last Updated on STN: 19971105  
Entered Medline: 19971023  
AB We have modified the automated **differential display** reverse transcription polymerase chain reaction technique (DDRT-PCR) such that a single fluorescently labeled universal primer (d(F)CTCACG-GATCCGTCGATT) is used in all PCRs together with a selection of arbitrary primers. We term this fluorescent detection procedure FDDRT-PCR. Anchoring primers of general structure dTGGTCTCACGGATCCTCGA-(T)12 VN (where N can be any deoxynucleoside and V can be any deoxynucleoside other than thymidine) are used for the RT step, and the universal primer together with selected arbitrary primers are then used for the PCR amplification. Advantages of this approach are: (i) the fluorescently labeled universal primer is a constant feature in every PCR, so that changes in banding profile are highly likely to reflect the incorporation of different arbitrary 10-mer primers; (ii) artifacts that result from arbitrary 10-mer to arbitrary 10-mer primer amplifications are

not observed by fluorescence detection on an automated gene scanner because such products are not fluorescently labeled; (iii) sample throughput and ease of data handling are increased when compared with the conventional radioactive/manual approach and (iv) using a single fluorescently labeled primer in all PCRs is highly cost-effective.

L4 ANSWER 8 OF 8 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN  
ACCESSION NUMBER: 97:113853 SCISEARCH  
THE GENUINE ARTICLE: WE924  
TITLE: DD/AP-PCR: Combination of **differential display** and arbitrarily primed PCR of oligo(dT) cDNA  
AUTHOR: Rothschild C B (Reprint); Brewer C S; Bowden D W  
CORPORATE SOURCE: WAKE FOREST UNIV, BOWMAN GRAY SCH MED, DEPT BIOCHEM,  
WINSTON SALEM, NC 27157 (Reprint)  
COUNTRY OF AUTHOR: USA  
SOURCE: ANALYTICAL BIOCHEMISTRY, (1 FEB 1997) Vol. 245, No. 1, pp.  
48-54.  
Publisher: ACADEMIC PRESS INC JNL-COMP SUBSCRIPTIONS, 525  
B ST, STE 1900, SAN DIEGO, CA 92101-4495.  
ISSN: 0003-2697.  
DOCUMENT TYPE: Article; Journal  
FILE SEGMENT: LIFE  
LANGUAGE: English  
REFERENCE COUNT: 11

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB In this report we describe the first direct comparison of **differential display** (DD) and arbitrarily primed PCR (AP-PGR) amplification of oligo(dT)-primed cDNA. Our results indicate that both of these widely used RNA fingerprinting techniques have their respective advantages and limitations. DD produces profiles specific to the anchored oligo(dT) primer used for cDNA synthesis. AP-PCR displays significant redundancy of profiles generated from different oligo(dT) cDNA pools, but is not as biased to the isolation of AT-rich or 3' sequences. It was found that both techniques can utilize cDNA synthesized using a generic anchored oligo(dT) primer (dT(12)**VN**; equimolar amounts of dT(12)VA, dT(12)VC, dT(12)VG, and dT(12)VT, where V is dA, dC, or dG); this efficiently selects for poly(A) (+) sequences from total RNA, and significantly reduces the number of cDNA preparations required per experiment. Using dT(12)**VN** cDNA pools generated from rat liver, spleen, and brain, the two approaches (AP-PCR and DD) were used in combination. Several known mRNAs were identified; some were unique to either technique and some were common to both. Since it is the RNA which is usually the limiting resource, maximum utilization may be achieved by generating a single pool of dT(12)**VN**-primed cDNA and performing both AP-PCR and DD (DD/AP-PCR). (C) 1997 Academic Press.

=> d his

(FILE 'HOME' ENTERED AT 13:51:22 ON 22 MAR 2005)

FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS'  
ENTERED AT 13:51:31 ON 22 MAR 2005

L1 13 S OLIGO-DT AND VN##  
L2 7 DUP REM L1 (6 DUPLICATES REMOVED)  
L3 20 S DIFFERENTIAL DISPLAY AND VN##  
L4 8 DUP REM L3 (12 DUPLICATES REMOVED)

=> s VNNN

L5 5 VNNN

=> d ibib abs 15 1-4

L5 ANSWER 1 OF 5 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN

ACCESSION NUMBER: 2002-17928 BIOTECHDS

TITLE: Identifying and characterizing gene expression in samples, for identifying mRNAs expressed at different levels, comprises employing an identimer having a oligo-dT primer of a specific sequence and a detectable marker at its 5' end; gene expression identification and characterization, database and computer bioinformatic software

AUTHOR: KANE M D; DOMBKOWSKI A A; NAGEL A C

PATENT ASSIGNEE: GENOMIC SOLUTIONS INC

PATENT INFO: WO 2002036828 10 May 2002

APPLICATION INFO: WO 2000-US45401 1 Nov 2000

PRIORITY INFO: US 2000-244933 1 Nov 2000

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2002-508123 [54]

AN 2002-17928 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - Systems for identification and characterization of gene expression in one or more samples, comprise an identimer having a specific oligo-dT primer sequence, where the identimer comprises a detectable marker at its 5' end.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a system (M1) for identification and characterization of gene expression in one or more samples, comprising: (a) providing one or more samples comprising one or more mRNA molecules; (b) providing an identimer comprising an oligo-dT primer of sequence, from 5' to 3' end, of (I) or (II), where the identimer also comprises a detectable marker at its 5' end; (c) contacting the mRNA with the identimer so that the polyT portion of the identimer hybridizes to the polyA tail of the mRNA and the VN<sub>x</sub> portion of the identimer hybridizes with portions of the mRNA immediately upstream of the polyA tail; (d) reverse transcribing the mRNA to produce a first strand cDNA that includes the identimer; (e) synthesizing a second DNA strand complementary to the first strand cDNA to form a duplex; (f) cleaving the duplex with a sequence-specific cleaving agent to provide one or more duplex cleavage fragments; (g) ligating an adaptamer comprising an RNA polymerase promoter site to one or more of the cleavage fragments; and (h) amplifying one or more ligated cleavage fragments using the identimer to produce one or more amplified fragments comprising sequences complementary to a 3' end of the mRNA; (2) a system for identification and characterization of gene expression in one or more samples, by: (a) employing steps (a) - (c) of M1; (b) providing a second sample comprising one or more mRNA molecules; (c) providing an identimer comprising (I) or (II); and (d) employing steps (c) - (h) of M1; (3) a system for identification and characterization of gene expression in one or more samples, by employing the steps of M1, and further contacting the in vitro transcribed RNA with the identimer so that the polyT portion of the identimer hybridizes to the polyA tail of the in vitro transcribed RNA and the (I) or (II) portion of the identimer hybridizes with portions of the in vitro transcribed RNA immediately upstream of the polyA tail, and reverse transcribing the in vitro transcribed RNA to produce a first strand cDNA that includes the identimer; (4) a system for identification and characterization of gene expression in two or more samples, comprising: (a) employing steps (a) - (c) of M1; (b) providing a second sample comprising one or more mRNA molecules; (c) providing an identimer comprising (I) or (II); (d) employing steps (d) - (h) of M1; (e) contacting the in vitro transcribed RNA with the identimer so that the polyT portion of the identimer hybridizes to the polyA tail of the in vitro transcribed RNA and the (I) or (II) portion of the identimer hybridizes with portions of the in vitro transcribed RNA immediately upstream of the polyA tail; and (f) reverse transcribing the in vitro transcribed RNA to produce a first strand cDNA that includes the identimer; (5) a kit comprising: (a) one or more

identimers comprising an oligo-dT primer of sequence, from 5' to 3' end, of (I), where the identimer also comprises a detectable marker at its 5' end; and (b) one or more sequence-specific cleaving agents. T<sub>n</sub>-VN<sub>x</sub> (I) T<sub>n</sub>-VNNN (II) n = an integer 8 or greater but not more than 50 representing the number of T's; V = a nucleotide a, c, or g but not t; N = a nucleotide a, c, g, or t; and x = an integer 3 or greater but not more than 10 representing the number of N nucleotides.

BIOTECHNOLOGY - Preferred Method: The system further comprises identifying and characterizing the cleavage fragments according to the presence of the marker, the sequences corresponding to the (I) or (II) nucleotide sequence and the sequence associated with the sequence-specific cleaving agent, and the size of the fragment. The system also includes identifying any gene associated with the cleavage fragments by comparing the sequence and size characteristics of the cleavage fragment with a database containing sequence and size characteristics of RNAs associated with known genes, where the comparison is conducted by means of software operated on a microprocessor.

USE - The system is useful for identifying any or all genes expressed in a given in vivo or in vitro RNA sample, as well as the relative differences in mRNA between 2 or more samples, where desired, for supporting discovery of new genes, and for identifying mRNAs that are expressed at different levels between 2 or more samples.

ADVANTAGE - The new system or method addresses limitations of prior methods by comprising compositions and systems that incorporate new strategies where molecular or biochemical assay compositions and systems are linked to DNA or RNA sequence databases for optimal resource efficiency in assaying gene expression. The system has the following advantages over prior and existing methods: (a) prior sequence information or clone library construction is not needed to enable the assay; (b) provides immediate sequence information in addition to information concerning changes or differences in mRNA level, to determine mRNA expression level and mRNA identification in one assay; (c) generates cDNA fragments from all mRNAs present in the sample for subsequent investigation by common molecular biology techniques; and (d) does not require prior knowledge of the sequence of the genome of the organism under investigation and can be employed in organisms lacking significant genomic sequence information.

EXAMPLE - Experimental protocols are described but no results were given. (45 pages)

L5 ANSWER 2 OF 5 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 84:266183 SCISEARCH

THE GENUINE ARTICLE: SR533

TITLE: ELECTRON-PARAMAGNETIC-RES INVESTIGATION OF THE INCOMMENSURATE PHASE OF THBR4 WITH THE HELP OF THE GD3& VNNN BR-

AUTHOR: EMERY J (Reprint); HUBERT S; FAYET J C

CORPORATE SOURCE: FAC SCI LE MANS, SPECTROSCOPIE SOLIDE LAB, ERA 682, F-72017 LE MANS, FRANCE (Reprint); INST PHYS NUCL, RADIOCHEM LAB, F-91406 ORSAY, FRANCE

COUNTRY OF AUTHOR: FRANCE

SOURCE: FERROELECTRICS, (1984) Vol. 53, No. 1-4, pp. 245.

DOCUMENT TYPE: Conference; Journal

FILE SEGMENT: PHYS

LANGUAGE: ENGLISH

REFERENCE COUNT: 3

L5 ANSWER 3 OF 5 CAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2002:353661 CAPLUS

DOCUMENT NUMBER: 136:351378

TITLE: Eukaryotic gene expression detection by reverse transcription with oligo-T primers and database searches

INVENTOR(S) : Kane, Michael David; Dombkowski, Alan A.; Nagel, Aaron C.  
 PATENT ASSIGNEE(S) : Genomic Solutions Inc., USA  
 SOURCE: PCT Int. Appl., 45 pp.  
 CODEN: PIXXD2  
 DOCUMENT TYPE: Patent  
 LANGUAGE: English  
 FAMILY ACC. NUM. COUNT: 1  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002036828	A2	20020510	WO 2001-US45401	20011101
WO 2002036828	A3	20030227		
W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG			
AU 2002018000	A5	20020515	AU 2002-18000	20011101
US 2003108874	A1	20030612	US 2001-2536	20011101
PRIORITY APPLN. INFO.:			US 2000-244933P	P 20001101
			WO 2001-US45401	W 20011101

**AB** The invention comprises compns. and systems to identify and compare expressed genes in a given in vivo or in vitro RNA sample, as well as the relative difference in mRNA expression between two or more sample, where desired. Furthermore, the invention comprises compns. and systems to identify novel genes. The invention comprises, without limitation, one or more mRNA specific identimers for use in reverse transcription that themselves comprise an oligo-T nucleotide sequence (at the 5' end) linked to a nucleotide sequence VN<sub>x</sub> (at the 3' end) where the V nucleotide immediately adjacent to the oligo-T segment is not a T. The present invention addresses limitations in the prior art by comprising compns. and systems that incorporate novel strategies whereby mol. or biochem. assay compns. and systems are linked to DNA or RNA sequence databases for optimal resource efficiency in assaying gene expression. Figure 1 shows a mol. protocol of one embodiment of the invention to generate 3' cDNA fragments for the assay of all polyadenylated mRNAs in eukaryotic samples. The sample under investigation is divided into 192 aliquots, and first strand synthesis (reverse transcription) is carried out using all **VNNN** combinations of the identimer, followed by second strand synthesis. The ds cDNA is cleaved in a sequence-specific manner using a restriction enzyme that involves a 4-base recognition sequence (e.g., Nla III). The resulting fragments are ligated to an adaptamer that contains one or more RNA polymerase promoter sites for subsequent in vitro transcription. The 3' cDNA fragments are initially enriched using PCR, primed at the adaptamer and the polyadenylation site (i.e. identimer), and subsequently employed as a template for in vitro transcription promoted within the adaptamer (e.g. T7 polymerase promoter in the ligated adaptamer). This results in an amplification of the sequence adjacent to, and downstream from, the RNA polymerase promoter sequence, which includes the restriction site and the polyadenylation site. "Second round" first strand synthesis is carried out using a fluorescence-labeled primer (identimer) to enable the detection of all 3' cDNA fragments for size and abundance (fluorescence label is denoted as an "\*" at the 5' end of the identimer in Figure 1). The entire process is repeated using a different restriction enzyme that employs a different recognition sequence (e.g. MboI). Gene (mRNA) identification is made by collecting knowledge of the 4 nucleotides upstream of the polyadenylation site (determined by identimer priming), the sequence of the restriction enzyme recognition site, and the

size of the fragment that provides the distance between the polyadenylation site and the proximal restriction site. This information is employed to search the known sequence database(s) to identify the mRNA(s) that match these criteria.

L5 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2005 ACS on STN  
ACCESSION NUMBER: 2001:365527 CAPLUS  
DOCUMENT NUMBER: 134:374588  
TITLE: Proposal of an extended t-J Hamiltonian for high-Tc cuprates from ab initio calculations on embedded clusters  
AUTHOR(S): Calzado, Carmen J.; Malrieu, Jean-Paul  
CORPORATE SOURCE: IRSAMC, Laboratoire de Physique Quantique, Universite Paul Sabatier, Toulouse, 31062, Fr.  
SOURCE: Physical Review B: Condensed Matter and Materials Physics (2001), 63(21), 214520/1-214520/13  
CODEN: PRBMDO; ISSN: 0163-1829  
PUBLISHER: American Physical Society  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB A series of accurate ab initio calcns. on CupOq finite clusters, properly embedded in the Madelung potential of the infinite lattice, have been performed in order to determine the local effective interactions in the CuO<sub>2</sub> planes of La<sub>2-x</sub>Sr<sub>x</sub>CuO<sub>4</sub> compds. The values of the first-neighbor interactions, magnetic coupling ( $J_{NN} = 125$  meV), and hopping integral ( $t_{NN} = -555$  meV) have been confirmed. Important addnl. effects are evidenced, concerning essentially the second-neighbor hopping integral  $t_{NNN} = +110$  meV, the displacement of a singlet toward an adjacent colinear hole,  $hSdabc = -80$  meV, a non-negligible hole-hole repulsion  $V_{NN}-V_{NNN}$  = 0.8 eV, and a strong anisotropic effect of the presence of an adjacent hole on the values of the first-neighbor interactions. The dependence of  $J_{NN}$  and  $t_{NN}$  on the position of neighbor hole(s) has been rationalized from the two-band model and checked from a series of addnl. ab initio calcns. An extended t-J model Hamiltonian has been proposed on the basis of these results. It is argued that the here-proposed three-body effects may play a role in the charge/spin separation observed in these compds., i.e., in the formation and dynamic of stripes.  
REFERENCE COUNT: 74 THERE ARE 74 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d his

(FILE 'HOME' ENTERED AT 13:51:22 ON 22 MAR 2005)

FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS'  
ENTERED AT 13:51:31 ON 22 MAR 2005

L1 13 S OLIGO-DT AND VN##  
L2 7 DUP REM L1 (6 DUPLICATES REMOVED)  
L3 20 S DIFFERENTIAL DISPLAY AND VN##  
L4 8 DUP REM L3 (12 DUPLICATES REMOVED)  
L5 5 S VNNN

=> s 15 5 ibib abs

MISSING OPERATOR L5 5 IBIB

The search profile that was entered contains terms or nested terms that are not separated by a logical operator.

=> d 15 5 ibib abs

L5 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2005 ACS on STN  
ACCESSION NUMBER: 2000:746459 CAPLUS  
DOCUMENT NUMBER: 134:35582  
TITLE: Proposal of an extended t-J Hamiltonian for high-Tc

cuprates from ab initio calculations on embedded clusters  
AUTHOR(S): Calzado, Carmen J.; Malrieu, Jean-Paul  
CORPORATE SOURCE: Laboratoire de Physique Quantique, IRSAMC, Universite Paul Sabatier, Toulouse, 31062, Fr.  
SOURCE: Los Alamos National Laboratory, Preprint Archive, Condensed Matter (2000) 1-28, arXiv:cond-mat/0010257, 18 Oct 2000  
CODEN: LNCMFR  
URL: <http://xxx.lanl.gov/pdf/cond-mat/0010257>  
PUBLISHER: Los Alamos National Laboratory  
DOCUMENT TYPE: Preprint  
LANGUAGE: English  
AB A series of accurate ab initio calcns. on Cu<sub>2</sub>O finite clusters, properly embedded on the Madelung potential of the infinite lattice, have been performed in order to determine the local effective interactions in the Cu<sub>2</sub>O planes of La<sub>2-x</sub>S<sub>x</sub>CuO<sub>4</sub> compds. The values of the first-neighbor interactions, magnetic coupling (JNN=125 meV) and hopping integral (tNN=-555 meV), have been confirmed. Important addnl. effects are evidenced, concerning essentially the second-neighbor hopping integral tNNN=+110meV, the displacement of a singlet toward an adjacent collinear hole, hSDabc=-80 meV, a non-negligible hole-hole repulsion VNN-VNNN=0.8 eV and a strong anisotropic effect of the presence of an adjacent hole on the values of the first-neighbor interactions. The dependence of JNN and tNN on the position of neighbor hole(s) has been rationalized from the two-band model and checked from a series of addnl. ab initio calcns. An extended t-J model Hamiltonian has been proposed on the basis of these results. It is argued that the here-proposed three-body effects may play a role in the charge/spin separation observed in these compds., i.e., in the formation and dynamic of stripes.  
REFERENCE COUNT: 57 THERE ARE 57 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d his

(FILE 'HOME' ENTERED AT 13:51:22 ON 22 MAR 2005)

FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS'  
ENTERED AT 13:51:31 ON 22 MAR 2005

L1 13 S OLIGO-DT AND VN##  
L2 7 DUP REM L1 (6 DUPLICATES REMOVED)  
L3 20 S DIFFERENTIAL DISPLAY AND VN##  
L4 8 DUP REM L3 (12 DUPLICATES REMOVED)  
L5 5 S VNNN

=> s primer and (VNNN### or VN1N2###)  
L6 2 PRIMER AND (VNNN### OR VN1N2###)

=> d ibib abs 16 1-2

L6 ANSWER 1 OF 2 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN  
ACCESSION NUMBER: 2002-17928 BIOTECHDS

TITLE: Identifying and characterizing gene expression in samples, for identifying mRNAs expressed at different levels, comprises employing an identimer having a oligo-dT primer of a specific sequence and a detectable marker at its 5' end; gene expression identification and characterization, database and computer bioinformatic software

AUTHOR: KANE M D; DOMBKOWSKI A A; NAGEL A C  
PATENT ASSIGNEE: GENOMIC SOLUTIONS INC  
PATENT INFO: WO 2002036828 10 May 2002

APPLICATION INFO: WO 2000-US45401 1 Nov 2000  
PRIORITY INFO: US 2000-244933 1 Nov 2000  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
OTHER SOURCE: WPI: 2002-508123 [54]  
AN 2002-17928 BIOTECHDS  
AB DERWENT ABSTRACT:

NOVELTY - Systems for identification and characterization of gene expression in one or more samples, comprise an identimer having a specific oligo-dT **primer** sequence, where the identimer comprises a detectable marker at its 5' end.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a system (M1) for identification and characterization of gene expression in one or more samples, comprising: (a) providing one or more samples comprising one or more mRNA molecules; (b) providing an identimer comprising an oligo-dT **primer** of sequence, from 5' to 3' end, of (I) or (II), where the identimer also comprises a detectable marker at its 5' end; (c) contacting the mRNA with the identimer so that the polyT portion of the identimer hybridizes to the polyA tail of the mRNA and the VN<sub>x</sub> portion of the identimer hybridizes with portions of the mRNA immediately upstream of the polyA tail; (d) reverse transcribing the mRNA to produce a first strand cDNA that includes the identimer; (e) synthesizing a second DNA strand complementary to the first strand cDNA to form a duplex; (f) cleaving the duplex with a sequence-specific cleaving agent to provide one or more duplex cleavage fragments; (g) ligating an adaptamer comprising an RNA polymerase promoter site to one or more of the cleavage fragments; and (h) amplifying one or more ligated cleavage fragments using the identimer to produce one or more amplified fragments comprising sequences complementary to a 3' end of the mRNA; (2) a system for identification and characterization of gene expression in one or more samples, by: (a) employing steps (a) - (c) of M1; (b) providing a second sample comprising one or more mRNA molecules; (c) providing an identimer comprising (I) or (II); and (d) employing steps (c)-(h) of M1; (3) a system for identification and characterization of gene expression in one or more samples, by employing the steps of M1, and further contacting the in vitro transcribed RNA with the identimer so that the polyT portion of the identimer hybridizes to the polyA tail of the in vitro transcribed RNA and the (I) or (II) portion of the identimer hybridizes with portions of the in vitro transcribed RNA immediately upstream of the polyA tail, and reverse transcribing the in vitro transcribed RNA to produce a first strand cDNA that includes the identimer; (4) a system for identification and characterization of gene expression in two or more samples, comprising: (a) employing steps (a) - (c) of M1; (b) providing a second sample comprising one or more mRNA molecules; (c) providing an identimer comprising (I) or (II); (d) employing steps (d) - (h) of M1; (e) contacting the in vitro transcribed RNA with the identimer so that the polyT portion of the identimer hybridizes to the polyA tail of the in vitro transcribed RNA and the (I) or (II) portion of the identimer hybridizes with portions of the in vitro transcribed RNA immediately upstream of the polyA tail; and (f) reverse transcribing the in vitro transcribed RNA to produce a first strand cDNA that includes the identimer; (5) a kit comprising: (a) one or more identimers comprising an oligo-dT **primer** of sequence, from 5' to 3' end, of (I), where the identimer also comprises a detectable marker at its 5' end; and (b) one or more sequence-specific cleaving agents.

Tn-VN<sub>x</sub> (I) Tn-VNNN (II) n = an integer 8 or greater but not more than 50 representing the number of T's; V = a nucleotide a, c, or g but not t; N = a nucleotide a, c, g, or t; and x = an integer 3 or greater but not more than 10 representing the number of N nucleotides.

BIOTECHNOLOGY - Preferred Method: The system further comprises identifying and characterizing the cleavage fragments according to the presence of the marker, the sequences corresponding to the (I) or (II) nucleotide sequence and the sequence associated with the sequence-specific cleaving agent, and the size of the fragment. The

system also includes identifying any gene associated with the cleavage fragments by comparing the sequence and size characteristics of the cleavage fragment with a database containing sequence and size characteristics of RNAs associated with known genes, where the comparison is conducted by means of software operated on a microprocessor.

USE - The system is useful for identifying any or all genes expressed in a given in vivo or in vitro RNA sample, as well as the relative differences in mRNA between 2 or more samples, where desired, for supporting discovery of new genes, and for identifying mRNAs that are expressed at different levels between 2 or more samples.

ADVANTAGE - The new system or method addresses limitations of prior methods by comprising compositions and systems that incorporate new strategies where molecular or biochemical assay compositions and systems are linked to DNA or RNA sequence databases for optimal resource efficiency in assaying gene expression. The system has the following advantages over prior and existing methods: (a) prior sequence information or clone library construction is not needed to enable the assay; (b) provides immediate sequence information in addition to information concerning changes or differences in mRNA level, to determine mRNA expression level and mRNA identification in one assay; (c) generates cDNA fragments from all mRNAs present in the sample for subsequent investigation by common molecular biology techniques; and (d) does not require prior knowledge of the sequence of the genome of the organism under investigation and can be employed in organisms lacking significant genomic sequence information.

EXAMPLE - Experimental protocols are described but no results were given. (45 pages)

L6 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2005 ACS on STN  
ACCESSION NUMBER: 2002:353661 CAPLUS  
DOCUMENT NUMBER: 136:351378  
TITLE: Eukaryotic gene expression detection by reverse transcription with oligo-T primers and database searches  
INVENTOR(S): Kane, Michael David; Dombkowski, Alan A.; Nagel, Aaron C.  
PATENT ASSIGNEE(S): Genomic Solutions Inc., USA  
SOURCE: PCT Int. Appl., 45 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002036828	A2	20020510	WO 2001-US45401	20011101
WO 2002036828	A3	20030227		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
AU 2002018000	A5	20020515	AU 2002-18000	20011101
US 2003108874	A1	20030612	US 2001-2536	20011101
PRIORITY APPLN. INFO.:			US 2000-244933P	P 20001101
			WO 2001-US45401	W 20011101

AB The invention comprises compositions and systems to identify and compare expressed genes in a given in vivo or in vitro RNA sample, as well as the relative difference in mRNA expression between two or more samples, where

desired. Furthermore, the invention comprises compns. and systems to identify novel genes. The invention comprises, without limitation, one or more mRNA specific identimers for use in reverse transcription that themselves comprise an oligo-T nucleotide sequence (at the 5' end) linked to a nucleotide sequence VN<sub>x</sub> (at the 3' end) where the V nucleotide immediately adjacent to the oligo-T segment is not a T. The present invention addresses limitations in the prior art by comprising compns. and systems that incorporate novel strategies whereby mol. or biochem. assay compns. and systems are linked to DNA or RNA sequence databases for optimal resource efficiency in assaying gene expression. Figure 1 shows a mol. protocol of one embodiment of the invention to generate 3' cDNA fragments for the assay of all polyadenylated mRNAs in eukaryotic samples. The sample under investigation is divided into 192 aliquots, and first strand synthesis (reverse transcription) is carried out using all VN<sub>NNN</sub> combinations of the identimer, followed by second strand synthesis. The ds cDNA is cleaved in a sequence-specific manner using a restriction enzyme that involves a 4-base recognition sequence (e.g., Nla III). The resulting fragments are ligated to an adaptamer that contains one or more RNA polymerase promoter sites for subsequent in vitro transcription. The 3' cDNA fragments are initially enriched using PCR, primed at the adaptamer and the polyadenylation site (i.e. identimer), and subsequently employed as a template for in vitro transcription promoted within the adaptamer (e.g. T7 polymerase promoter in the ligated adaptamer). This results in an amplification of the sequence adjacent to, and downstream from, the RNA polymerase promoter sequence, which includes the restriction site and the polyadenylation site. "Second round" first strand synthesis is carried out using a fluorescence-labeled primer (identimer) to enable the detection of all 3' cDNA fragments for size and abundance (fluorescence label is denoted as an "\*" at the 5' end of the identimer in Figure 1). The entire process is repeated using a different restriction enzyme that employs a different recognition sequence (e.g. MboI). Gene (mRNA) identification is made by collecting knowledge of the 4 nucleotides upstream of the polyadenylation site (determined by identimer priming), the sequence of the restriction enzyme recognition site, and the size of the fragment that provides the distance between the polyadenylation site and the proximal restriction site. This information is employed to search the known sequence database(s) to identify the mRNA(s) that match these criteria.

=> d his

(FILE 'HOME' ENTERED AT 13:51:22 ON 22 MAR 2005)

FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS'  
ENTERED AT 13:51:31 ON 22 MAR 2005

L1           13 S OLIGO-DT AND VN##  
L2           7 DUP REM L1 (6 DUPLICATES REMOVED)  
L3           20 S DIFFERENTIAL DISPLAY AND VN##  
L4           8 DUP REM L3 (12 DUPLICATES REMOVED)  
L5           5 S VN<sub>NNN</sub>  
L6           2 S PRIMER AND (VN<sub>NNN</sub>## OR VN1N2##)

=> s vn1n2#

L7           0 VN1N2#

=> identimer

IDENTIMER IS NOT A RECOGNIZED COMMAND

The previous command name entered was not recognized by the system.  
For a list of commands available to you in the current file, enter  
"HELP COMMANDS" at an arrow prompt (>).

=> s identimer

L8           2 IDENTIMER

=> d ibib abs 18 1-2

L8 ANSWER 1 OF 2 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN  
ACCESSION NUMBER: 2002-17928 BIOTECHDS  
TITLE: Identifying and characterizing gene expression in samples,  
for identifying mRNAs expressed at different levels,  
comprises employing an **identimer** having a oligo-dT  
primer of a specific sequence and a detectable marker at its  
5' end;  
gene expression identification an characterization,  
database and computer bioinformatic software  
AUTHOR: KANE M D; DOMBKOWSKI A A; NAGEL A C  
PATENT ASSIGNEE: GENOMIC SOLUTIONS INC  
PATENT INFO: WO 2002036828 10 May 2002  
APPLICATION INFO: WO 2000-US45401 1 Nov 2000  
PRIORITY INFO: US 2000-244933 1 Nov 2000  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
OTHER SOURCE: WPI: 2002-508123 [54]  
AN 2002-17928 BIOTECHDS  
AB DERWENT ABSTRACT:  
NOVELTY - Systems for identification and characterization of gene  
expression in one or more samples, comprise an **identimer** having  
a specific oligo-dT primer sequence, where the **identimer**  
comprises a detectable marker at its 5' end.  
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the  
following: (1) a system (M1) for identification and characterization of  
gene expression in one or more samples, comprising: (a) providing one or  
more samples comprising one or more mRNA molecules; (b) providing an  
**identimer** comprising an oligo-dT primer of sequence, from 5' to  
3' end, of (I) or (II), where the **identimer** also comprises a  
detectable marker at its 5' end; (c) contacting the mRNA with the  
**identimer** so that the polyT portion of the **identimer**  
hybridizes to the polyA tail of the mRNA and the VN<sub>x</sub> portion of the  
**identimer** hybridizes with portions of the mRNA immediately  
upstream of the polyA tail; (d) reverse transcribing the mRNA to produce  
a first strand cDNA that includes the **identimer**; (e)  
synthesizing a second DNA strand complementary to the first strand cDNA  
to form a duplex; (f) cleaving the duplex with a sequence-specific  
cleaving agent to provide one or more duplex cleavage fragments; (g)  
ligating an adaptamer comprising an RNA polymerase promoter site to one  
or more of the cleavage fragments; and (h) amplifying one or more ligated  
cleavage fragments using the **identimer** to produce one or more  
amplified fragments comprising sequences complementary to a 3' end of the  
mRNA; (2) a system for identification and characterization of gene  
expression in one or more samples, by: (a) employing steps (a) - (c) of  
M1; (b) providing a second sample comprising one or more mRNA molecules;  
(c) providing an **identimer** comprising (I) or (II); and (d)  
employing steps (c)-(h) of M1; (3) a system for identification and  
characterization of gene expression in one or more samples, by employing  
the steps of M1, and further contacting the in vitro transcribed RNA with  
the **identimer** so that the polyT portion of the  
**identimer** hybridizes to the polyA tail of the in vitro  
transcribed RNA and the (I) or (II) portion of the **identimer**  
hybridizes with portions of the in vitro transcribed RNA immediately  
upstream of the polyA tail, and reverse transcribing the in vitro  
transcribed RNA to produce a first strand cDNA that includes the  
**identimer**; (4) a system for identification and characterization  
of gene expression in two or more samples, comprising: (a) employing  
steps (a) - (c) of M1; (b) providing a second sample comprising one or  
more mRNA molecules; (c) providing an **identimer** comprising (I)  
or (II); (d) employing steps (d) - (h) of M1; (e) contacting the in vitro  
transcribed RNA with the **identimer** so that the polyT portion of

the **identimer** hybridizes to the polyA tail of the in vitro transcribed RNA and the (I) or (II) portion of the **identimer** hybridizes with portions of the in vitro transcribed RNA immediately upstream of the polyA tail; and (f) reverse transcribing the in vitro transcribed RNA to produce a first strand cDNA that includes the **identimer**; (5) a kit comprising: (a) one or more identimers comprising an oligo-dT primer of sequence, from 5' to 3' end, of (I), where the **identimer** also comprises a detectable marker at its 5' end; and (b) one or more sequence-specific cleaving agents. Tn-VNx (I) Tn-VNNN (II) n = an integer 8 or greater but not more than 50 representing the number of T's; V = a nucleotide a, c, or g but not t; N = a nucleotide a, c, g, or t; and x = an integer 3 or greater but not more than 10 representing the number of N nucleotides.

**BIOTECHNOLOGY - Preferred Method:** The system further comprises identifying and characterizing the cleavage fragments according to the presence of the marker, the sequences corresponding to the (I) or (II) nucleotide sequence and the sequence associated with the sequence-specific cleaving agent, and the size of the fragment. The system also includes identifying any gene associated with the cleavage fragments by comparing the sequence and size characteristics of the cleavage fragment with a database contacting sequence and size characteristics of RNAs associated with known genes, where the comparison is conducted by means of software operated on a microprocessor.

**USE -** The system is useful for identifying any or all genes expressed in a given in vivo or in vitro RNA sample, as well as the relative differences in mRNA between 2 or more samples, where desired, for supporting discovery of new genes, and for identifying mRNAs that are expressed at different levels between 2 or more samples.

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**EXAMPLE -** Experimental protocols are described but no results were given. (45 pages)

L8 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2005 ACS on STN  
ACCESSION NUMBER: 2002:353661 CAPLUS  
DOCUMENT NUMBER: 136:351378  
TITLE: Eukaryotic gene expression detection by reverse transcription with oligo-T primers and database searches  
INVENTOR(S): Kane, Michael David; Dombkowski, Alan A.; Nagel, Aaron C.  
PATENT ASSIGNEE(S): Genomic Solutions Inc., USA  
SOURCE: PCT Int. Appl., 45 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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WO 2002036828	A2	20020510	WO 2001-US45401	20011101
WO 2002036828	A3	20030227		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
AU 2002018000	A5	20020515	AU 2002-18000	20011101
US 2003108874	A1	20030612	US 2001-2536	20011101
PRIORITY APPLN. INFO.: US 2000-244933P P 20001101 WO 2001-US45401 W 20011101				

AB The invention comprises compns. and systems to identify and compare expressed genes in a given in vivo or in vitro RNA sample, as well as the relative difference in mRNA expression between two or more sample, where desired. Furthermore, the invention comprises compns. and systems to identify novel genes. The invention comprises, without limitation, one or more mRNA specific identimers for use in reverse transcription that themselves comprise an oligo-T nucleotide sequence (at the 5' end) linked to a nucleotide sequence VN<sub>x</sub> (at the 3' end) where the V nucleotide immediately adjacent to the oligo-T segment is not a T. The present invention addresses limitations in the prior art by comprising compns. and systems that incorporate novel strategies whereby mol. or biochem. assay compns. and systems are linked to DNA or RNA sequence databases for optimal resource efficiency in assaying gene expression. Figure 1 shows a mol. protocol of one embodiment of the invention to generate 3' cDNA fragments for the assay of all polyadenylated mRNAs in eukaryotic samples. The sample under investigation is divided into 192 aliquots, and first strand synthesis (reverse transcription) is carried out using all VNNN combinations of the **identimer**, followed by second strand synthesis. The ds cDNA is cleaved in a sequence-specific manner using a restriction enzyme that involves a 4-base recognition sequence (e.g., Nla III). The resulting fragments are ligated to an adaptamer that contains one or more RNA polymerase promoter sites for subsequent in vitro transcription. The 3' cDNA fragments are initially enriched using PCR, primed at the adaptamer and the polyadenylation site (i.e. **identimer**), and subsequently employed as a template for in vitro transcription promoted within the adaptamer (e.g. T7 polymerase promoter in the ligated adaptamer). This results in an amplification of the sequence adjacent to, and downstream from, the RNA polymerase promoter sequence, which includes the restriction site and the polyadenylation site. "Second round" first strand synthesis is carried out using a fluorescence-labeled primer (**identimer**) to enable the detection of all 3' cDNA fragments for size and abundance (fluorescence label is denoted as an "\*" at the 5' end of the **identimer** in Figure 1). The entire process is repeated using a different restriction enzyme that employs a different recognition sequence (e.g. MboI). Gene (mRNA) identification is made by collecting knowledge of the 4 nucleotides upstream of the polyadenylation site (determined by **identimer** priming), the sequence of the restriction enzyme recognition site, and the size of the fragment that provides the distance between the polyadenylation site and the proximal restriction site. This information is employed to search the known sequence database(s) to identify the mRNA(s) that match these criteria.

=> s VN? and permutation  
L9 19 VN? AND PERMUTATION

=> dup rem 19  
PROCESSING COMPLETED FOR L9

L10

7 DUP REM L9 (12 DUPLICATES REMOVED)

=> d ibib abs 110 1-10

L10 ANSWER 1 OF 7 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN  
ACCESSION NUMBER: 2004:852766 SCISEARCH  
THE GENUINE ARTICLE: BAV32  
TITLE: Particle swarm optimization algorithm for permutation flowshop sequencing problem  
AUTHOR: Tasgetiren M F (Reprint); Sevkli M; Liang Y C; Gencyilmaz G  
CORPORATE SOURCE: Fatih Univ, Dept Management, TR-34500 Istanbul, Turkey (Reprint); Fatih Univ, Dept Ind Engn, TR-34500 Istanbul, Turkey; Yuan Ze Univ, Dept Ind Engn & Management, Taoyuan 320, Taiwan; Istanbul Kultur Univ, Dept Management, Istanbul, Turkey  
COUNTRY OF AUTHOR: Turkey; Taiwan  
SOURCE: ANT COLONY OPTIMIZATION AND SWARM INTELLIGENCE, PROCEEDINGS, (OCT 2004) Vol. 3172, pp. 382-389.  
Publisher: SPRINGER-VERLAG BERLIN, HEIDELBERGER PLATZ 3, D-14197 BERLIN, GERMANY.  
ISSN: 0302-9743.  
DOCUMENT TYPE: Article; Journal  
LANGUAGE: English  
REFERENCE COUNT: 20

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB This paper presents a particle swarm optimization algorithm (PSO) to solve the **permutation** flowshop sequencing problem (PFSP) with makespan criterion. Simple but very efficient local search based on the variable neighborhood search (**VNS**) is embedded in the PSO algorithm to solve the benchmark suites in the literature. The results are presented and compared to the best known approaches in the literature. Ultimately, a total of 195 out of 800 best-known solutions in the literature is improved by the **VNS** version of the PSO algorithm.

L10 ANSWER 2 OF 7 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN  
DUPLICATE 1  
ACCESSION NUMBER: 2002:294993 BIOSIS  
DOCUMENT NUMBER: PREV200200294993  
TITLE: Genetic diversity among populations and size classes of buckeyes (*Aesculus: Hippocastanaceae*) examined with multilocus **VNTR** probes.  
AUTHOR(S): Lim, H. W. [Reprint author]; Pelikan, S. [Reprint author]; Rogstad, S. H. [Reprint author]  
CORPORATE SOURCE: Biological Sciences, University of Cincinnati, ML0006, Cincinnati, OH, 45221-0006, USA  
steven.rogstad@uc.edu  
SOURCE: Plant Systematics and Evolution, (2002) Vol. 230, No. 3-4, pp. 125-141. print.  
CODEN: ESPFBP. ISSN: 0378-2697.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
ENTRY DATE: Entered STN: 15 May 2002  
Last Updated on STN: 15 May 2002

AB Little is known about genetic variation in members of the genus *Aesculus* (Hippocastanaceae), in particular *A. flava* (yellow buckeye) and *A. glabra* (Ohio buckeye). Here, three synthetic DNA probes (composed of tandemly repeated, core sequences) that reveal alleles at multiple variable-number tandem-repeat (**VNTR**) loci in these two species were used to investigate: 1) levels of genetic variation in one stand of *A. flava* and three isolated stands of *A. glabra*; 2) whether the stands of *A. glabra* are genetically differentiated from one another; 3) whether there has been selection for more heterozygous individuals through time in one stand each

of *A. flava* and *A. glabra*; and 4) whether a possible genetic bottleneck had occurred during the formation of either species of *Aesculus*. First, variation of **VNTR** genetic markers within and among three populations of *A. glabra* separated by 60-180 km was examined. In each one hectare (ha) population, 22 individuals were randomly sampled. Among the three populations, the mean number of bands scored per individual was 80.35 and the average number of estimated loci surveyed was 54.17. Mean similarity and estimated heterozygosity within populations ranged from 0.634 to 0.743 and from 0.342 to 0.486, respectively. The mean similarity across populations was 0.657, while the mean estimated heterozygosity across populations was 0.484 for *A. glabra*. The most isolated site was the most genetically differentiated as indicated by differences in levels of similarity, heterozygosity, and Fst value comparisons. In a separate experiment, genetic variation in 22 large (reproductively mature; dbh > 8 cm) individuals was compared with that in 22 small (not yet reproductive; dbh < 1 cm) individuals collected within one ha stands for both *A. flava* and *A. glabra*. Mean similarity values among large versus small individuals of *A. flava* were 0.665 versus 0.662, while for *A. glabra* the corresponding values were 0.686 versus 0.691, respectively.

**Permutation** tests of these similarity data detected no evidence for size class genetic differentiation in either species (both p-values > 0.050). Further, **permutation** tests for the number of bands per individual (average band number should be higher in more heterozygous individuals) detected no significant differences between size classes for either species. Thus, evidence of pronounced inbreeding and/or selection altering population genetics within small relative to large individuals was not detected. In addition, comparable similarity and heterozygosity values between these two closely related species (which still maintain an active zone of hybridization) suggests that either: 1) no extreme genetic bottleneck has accompanied the formation of these species from a common ancestor; or 2) signs of such a bottleneck have largely been eliminated. These studies demonstrate the utility of multilocus **VNTR** DNA probes for investigating genetic variation within and among plant populations, between size classes within a population, and between closely related species.

L10 ANSWER 3 OF 7 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN  
ACCESSION NUMBER: 2002:9602 SCISEARCH  
THE GENUINE ARTICLE: 502JE  
TITLE: Linear maps leaving the alternating group invariant  
AUTHOR: Chiang H; Li C K (Reprint)  
CORPORATE SOURCE: Coll William & Mary, Dept Math, POB 8795, Williamsburg, VA 23187 USA (Reprint); Coll William & Mary, Dept Math, Williamsburg, VA 23187 USA  
COUNTRY OF AUTHOR: USA  
SOURCE: LINEAR ALGEBRA AND ITS APPLICATIONS, (1 JAN 2002) Vol. 340, pp. 69-80.  
Publisher: ELSEVIER SCIENCE INC, 655 AVENUE OF THE AMERICAS, NEW YORK, NY 10010 USA.  
ISSN: 0024-3795.  
DOCUMENT TYPE: Article; Journal  
LANGUAGE: English  
REFERENCE COUNT: 3

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Let  $A(n)$  be the group of  $n \times n$  even **permutation** matrices, and let  $V_n$  be the real linear space spanned by  $A(n)$ . The purpose of this note is to characterize those linear operators  $\phi$  on  $V_n$  satisfying  $\phi(A(n)) = A(n)$ . This answers a question raised by C.K. Li, B.S. Tam, N.K. Tsing [Linear Algebra Appl., to appear]. (C) 2002 Elsevier Science Inc. All rights reserved.

L10 ANSWER 4 OF 7 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN  
DUPLICATE 2

ACCESSION NUMBER: 1999:493524 BIOSIS  
DOCUMENT NUMBER: PREV199900493524  
TITLE: Genetic diversity of *Typha latifolia* (Typhaceae) and the impact of pollutants examined with tandem-repetitive DNA probes.  
AUTHOR(S): Keane, Brian; Pelikan, Stephan; Toth, Greg P.; Smith, M. Kate; Rogstad, Steven H. [Reprint author]  
CORPORATE SOURCE: Department of Biological Sciences ML6, University of Cincinnati, Cincinnati, OH, 45221-0006, USA  
SOURCE: American Journal of Botany, (Sept., 1999) Vol. 86, No. 9, pp. 1226-1238. print.  
CODEN: AJBOAA. ISSN: 0002-9122.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
ENTRY DATE: Entered STN: 16 Nov 1999  
Last Updated on STN: 16 Nov 1999

AB Genetic diversity at variable-number-tandem-repeat (**VNTR**) loci was examined in the common cattail, *Typha latifolia* (Typhaceae), using three synthetic DNA probes composed of tandemly repeated "core" sequences (GACA, GATA, and GCAC). The principal objectives of this investigation were to determine whether: (1) the previously reported almost complete lack of polymorphism at allozyme loci in this species was indicative of a reduced amount of genetic diversity at **VNTR** loci as well; (2) **VNTR** markers were informative about possible clonal propagation; and (3) significant differences in genetic structure of sampling sites were associated with differences in environmental levels of pollutants at those sites. Previously, widespread sampling across the eastern United States, surveying across ten allozyme loci, has detected only two genotypes, involving a difference at a single locus, among 104 populations. In this study, the amount of genetic diversity detected at **VNTR** loci: (1) among ramets (N= 40; 40 genotypes detected) collected at apprx8-km intervals along a 320-km transect; (2) among ramets (N = 220; 117 genotypes detected) from five study sites separated by 50-3000 m; and (3) even among ramets within each study site (N = 44 per site; from 13 to 34 genotypes detected per site (270 m<sup>2</sup>)) exceeds that previously found in those more geographically widespread allozyme surveys. Among the 260 ramets analyzed here, the mean number of bands scored per individual was 48.61 (SD = 2.80). Mean genetic similarity among ramets collected along the 320-km transect was 0.91, which was within the range of mean genetic similarity within the five study sites (range: 0.89-0.95). Among the five study sites, 61% of the samples analyzed appeared to be clonal ramets, with up to 12 clones detected for 44 ramets sampled within a site. Clones grew intermingled and ranged up to 39 m in extent.

**Permutation** tests of genetic similarity revealed significant genetic differentiation between each of the five study sites. Consistent with the previous allozyme studies, *T. latifolia* was characterized by extremely low genetic variation relative to levels of polymorphism detected at **VNTR** loci in other plant species. Estimated heterozygosity among ramets along the 320-km transect ranged from 0.11 to 0.13, while that within the five study sites ranged from 0.05 to 0.12. Estimates of Fst (0.32-0.41) also indicated considerable genetic subdivision among these stands. Significantly higher genetic diversity was detected at the two study sites that chemistry and toxicity data indicate to be the most severely impacted by pollutants. Although this correlation does not establish cause and effect, the results of this study indicate that the analysis of genetic diversity at **VNTR** loci may be a useful tool for monitoring anthropogenic-induced changes in the genetic structure of natural populations of plants.

L10 ANSWER 5 OF 7 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN  
DUPLICATE 3  
ACCESSION NUMBER: 1998:93708 BIOSIS  
DOCUMENT NUMBER: PREV199800093708  
TITLE: Genetic diversity of Philippine *Rubus moluccanus* L.

(Rosaceae) populations examined with **VNTR** DNA probes.  
AUTHOR(S): Busemeyer, Daniel T.; Pelikan, Stephan; Kennedy, Robert S.;  
Rogstad, Steven H. [Reprint author]  
CORPORATE SOURCE: Dep. Biol. Sci. ML6, Univ. Cincinnati, Cincinnati, OH  
45221-0006, USA  
SOURCE: Journal of Tropical Ecology, (Nov., 1997) Vol. 13, No. 6,  
pp. 867-884. print.  
ISSN: 0266-4674.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
ENTRY DATE: Entered STN: 25 Feb 1998  
Last Updated on STN: 25 Feb 1998

AB Two synthetic DNA probes composed of tandemly repeated 'core' sequences (GACA and GATA) were used to examine genetic diversity at multiple variable-number-tandem-repeat (**VNTR**) loci within and among four spatially isolated Philippine populations of *Rubus moluccanus* L. (Rosaceae), an uncultivated bramble widely distributed throughout southeast Asia - Malesia. Central goals were to determine whether apomictic propagation was detectable in *R. moluccanus* and to examine whether populations isolated on separate islands, or on mountain tops on a single island, were genetically differentiated. Sampling 22 individuals per population, the findings include: (1) no two individuals shared identical **VNTR** band profiles and thus apomictic propagation was not detected; (2) the mean number of bands scored per individual was 24.3 (SD = 3.91) and the proportion of polymorphic loci within populations ranged from 0.69 to 1.00 (mean = 0.86 +- SD = 0.085); (3) the average proportion of bands shared between individuals within populations ranged from 0.39 to 0.67 (0.50 +- 0.067), while average interpopulation similarity ranged from 0.21 to 0.50 (0.32 +- 0.092); and (4) estimated heterozygosity within populations ranged from 0.42 to 0.79 (0.62 +- 0.083), while interpopulation heterozygosity ran from 0.62 to 0.81 (0.74 +- 0.062). **Permutation** tests were used to estimate the statistical significance of differences in similarity between populations. A Luzon population isolated by 1200 km was always significantly different in similarity tests when compared with each of the other three populations, all located on Mindanao (and separated by at least 100 km but less than 250 km). Of the latter three, only one population differed significantly from the other two in the degree of **VNTR** markers shared, possibly reflecting biogeographic partitioning suggested for the island. Across all populations, average estimated Fst was 0.154, although mean inter-island Fst (0.224) was significantly higher than mean intra-island Fst (0.085). These results suggest that, while intra-island gene flow is relatively high, significant differentiation of tropical species may occur even over short distances on individual islands. Preservation of only a limited number of populations may result in a significant loss of genetic diversity in such species.

L10 ANSWER 6 OF 7 MEDLINE on STN DUPLICATE 4  
ACCESSION NUMBER: 97124707 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 8969844  
TITLE: GELSTATS: a computer program for population genetics analyses using **VNTR** multilocus probe data.  
AUTHOR: Rogstad S H; Pelikan S  
CORPORATE SOURCE: University of Cincinnati, OH, USA..  
rogstad@ucbeh.san.uc.edu  
SOURCE: BioTechniques, (1996 Dec) 21 (6) 1128-31.  
Journal code: 8306785. ISSN: 0736-6205.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199703  
ENTRY DATE: Entered STN: 19970321

Last Updated on STN: 19970321

Entered Medline: 19970311

AB GELSTATS, a computer program for population genetics analyses utilizing genetic markers revealed with variable number tandem repeat (**VNTR**) multilocus probes, is described and made available (both as C++ source code and as an executable DOS program). The program calculates several population genetics parameters, including: (i) individual and population band numbers; (ii) population bands exhibiting complete linkage (redundant examples of such bands can be removed in subsequent analyses); (iii) similarity (fraction of bands shared) between individuals and average similarity within and between designated groups; (iv) estimated probability that two individuals chosen at random will have identical band profiles; (v) heterozygosity estimates for designated groups; and (vi) Fst estimates. Nonparametric **permutation** methods are used to assess the significance of differences in both within- and between-group similarity. A jackknife test for heterozygosity differences between groups is also computed. Examples of GELSTATS analyses illustrate some features of the program.

L10 ANSWER 7 OF 7 MEDLINE on STN DUPLICATE 5  
ACCESSION NUMBER: 93170644 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 8094698  
TITLE: Intraclass and interclass correlations of allele sizes within and between loci in DNA typing data.  
AUTHOR: Chakraborty R; Srinivasan M R; de Andrade M  
CORPORATE SOURCE: Center for Demographic and Population Genetics, University of Texas Graduate School of Biomedical Sciences, Houston 77225.  
CONTRACT NUMBER: GM-41399 (NIGMS)  
SOURCE: Genetics, (1993 Feb) 133 (2) 411-9.  
Journal code: 0374636. ISSN: 0016-6731.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199303  
ENTRY DATE: Entered STN: 19930402  
Last Updated on STN: 19950206  
Entered Medline: 19930323

AB Nonparametric measures of correlations of DNA fragment lengths within and between variable number of tandem repeat (**VNTR**) loci are proposed to test the hypothesis of random association of allele sizes at **VNTR** loci. Transformations of these nonparametric correlation measures are suggested to detect deviations of their null expectations caused by population subdivision and errors of measurement of **VNTR** fragment lengths. Analytic and **permutation**-based computer simulation studies are performed to show that under the hypothesis of independence of allele sizes the transformed correlation measures are normally distributed, irrespective of the **VNTR** fragment size distribution in the population even when the number of individuals samples is as low as 100. Power calculations are performed to establish that the current population data on six **VNTR** loci in the US Hispanic sample are in accordance with the hypothesis of random association of allele sizes within and between loci. Implications of these results in the context of forensic use of DNA typing are also discussed.

=> d his

(FILE 'HOME' ENTERED AT 13:51:22 ON 22 MAR 2005)

FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS'  
ENTERED AT 13:51:31 ON 22 MAR 2005

L1 13 S OLIGO-DT AND VN##

L2            7 DUP REM L1 (6 DUPLICATES REMOVED)  
L3            20 S DIFFERENTIAL DISPLAY AND VN###  
L4            8 DUP REM L3 (12 DUPLICATES REMOVED)  
L5            5 S VNNN  
L6            2 S PRIMER AND (VNNN### OR VN1N2###)  
L7            0 S VN1N2#  
L8            2 S IDENTIMER  
L9            19 S VN? AND PERMUTATION  
L10          7 DUP REM L9 (12 DUPLICATES REMOVED)

=> S VN<sub>x</sub>  
L11          185 VNX

=> S l11 and oligo-dt  
3 FILES SEARCHED...  
L12          1 L11 AND OLIGO-DT

=> d all

L12          ANSWER 1 OF 1 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN  
AN          2002-17928 BIOTECHDS  
TI          Identifying and characterizing gene expression in samples, for  
identifying mRNAs expressed at different levels, comprises employing an  
identimer having a **oligo-dT** primer of a specific  
sequence and a detectable marker at its 5' end;  
gene expression identification and characterization, database and  
computer bioinformatic software  
AU          KANE M D; DOMBKOWSKI A A; NAGEL A C  
PA          GENOMIC SOLUTIONS INC  
PI          WO 2002036828 10 May 2002  
AI          WO 2000-US45401 1 Nov 2000  
PRAI        US 2000-244933 1 Nov 2000  
DT          Patent  
LA          English  
OS          WPI: 2002-508123 [54]  
AB          DERWENT ABSTRACT:  
NOVELTY - Systems for identification and characterization of gene  
expression in one or more samples, comprise an identimer having a  
specific **oligo-dT** primer sequence, where the  
identimer comprises a detectable marker at its 5' end.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the  
following: (1) a system (M1) for identification and characterization of  
gene expression in one or more samples, comprising: (a) providing one or  
more samples comprising one or more mRNA molecules; (b) providing an  
identimer comprising an **oligo-dT** primer of sequence,  
from 5' to 3' end, of (I) or (II), where the identimer also comprises a  
detectable marker at its 5' end; (c) contacting the mRNA with the  
identimer so that the polyT portion of the identimer hybridizes to the  
polyA tail of the mRNA and the **VNx** portion of the identimer  
hybridizes with portions of the mRNA immediately upstream of the polyA  
tail; (d) reverse transcribing the mRNA to produce a first strand cDNA  
that includes the identimer; (e) synthesizing a second DNA strand  
complementary to the first strand cDNA to form a duplex; (f) cleaving the  
duplex with a sequence-specific cleaving agent to provide one or more  
duplex cleavage fragments; (g) ligating an adaptamer comprising an RNA  
polymerase promoter site to one or more of the cleavage fragments; and  
(h) amplifying one or more ligated cleavage fragments using the identimer  
to produce one or more amplified fragments comprising sequences  
complementary to a 3' end of the mRNA; (2) a system for identification  
and characterization of gene expression in one or more samples, by: (a)  
employing steps (a) - (c) of M1; (b) providing a second sample comprising  
one or more mRNA molecules; (c) providing an identimer comprising (I) or  
(II); and (d) employing steps (c)-(h) of M1; (3) a system for  
identification and characterization of gene expression in one or more

samples, by employing the steps of M1, and further contacting the in vitro transcribed RNA with the identimer so that the polyT portion of the identimer hybridizes to the polyA tail of the in vitro transcribed RNA and the (I) or (II) portion of the identimer hybridizes with portions of the in vitro transcribed RNA immediately upstream of the polyA tail, and reverse transcribing the in vitro transcribed RNA to produce a first strand cDNA that includes the identimer; (4) a system for identification and characterization of gene expression in two or more samples, comprising: (a) employing steps (a) - (c) of M1; (b) providing a second sample comprising one or more mRNA molecules; (c) providing an identimer comprising (I) or (II); (d) employing steps (d) - (h) of M1; (e) contacting the in vitro transcribed RNA with the identimer so that the polyT portion of the identimer hybridizes to the polyA tail of the in vitro transcribed RNA and the (I) or (II) portion of the identimer hybridizes with portions of the in vitro transcribed RNA immediately upstream of the polyA tail; and (f) reverse transcribing the in vitro transcribed RNA to produce a first strand cDNA that includes the identimer; (5) a kit comprising: (a) one or more identimers comprising an **oligo-dT** primer of sequence, from 5' to 3' end, of (I), where the identimer also comprises a detectable marker at its 5' end; and (b) one or more sequence-specific cleaving agents. Tn-VNx (I)  
Tn-VNNN (II) n = an integer 8 or greater but not more than 50  
representing the number of T's; V = a nucleotide a, c, or g but not t; N = a nucleotide a, c, g, or t; and x = an integer 3 or greater but not more than 10 representing the number of N nucleotides.

**BIOTECHNOLOGY - Preferred Method:** The system further comprises identifying and characterizing the cleavage fragments according to the presence of the marker, the sequences corresponding to the (I) or (II) nucleotide sequence and the sequence associated with the sequence-specific cleaving agent, and the size of the fragment. The system also includes identifying any gene associated with the cleavage fragments by comparing the sequence and size characteristics of the cleavage fragment with a database contacting sequence and size characteristics of RNAs associated with known genes, where the comparison is conducted by means of software operated on a microprocessor.

**USE -** The system is useful for identifying any or all genes expressed in a given in vivo or in vitro RNA sample, as well as the relative differences in mRNA between 2 or more samples, where desired, for supporting discovery of new genes, and for identifying mRNAs that are expressed at different levels between 2 or more samples.

**ADVANTAGE -** The new system or method addresses limitations of prior methods by comprising compositions and systems that incorporate new strategies where molecular or biochemical assay compositions and systems are linked to DNA or RNA sequence databases for optimal resource efficiency in assaying gene expression. The system has the following advantages over prior and existing methods: (a) prior sequence information or clone library construction is not needed to enable the assay; (b) provides immediate sequence information in addition to information concerning changes or differences in mRNA level, to determine mRNA expression level and mRNA identification in one assay; (c) generates cDNA fragments from all mRNAs present in the sample for subsequent investigation by common molecular biology techniques; and (d) does not require prior knowledge of the sequence of the genome of the organism under investigation and can be employed in organisms lacking significant genomic sequence information.

**EXAMPLE -** Experimental protocols are described but no results were given. (45 pages)

CC GENETIC TECHNIQUES and APPLICATIONS, Gene Expression Techniques and Analysis; BIOINFORMATICS and ANALYSIS, Software; BIOINFORMATICS and ANALYSIS, Databases  
CT GENE EXPRESSION IDENTIFICATION, CHARACTERIZATION, IDENTIMER, OLIGO-DT DNA PRIMER SEQUENCE, DETECTABLE MARKER, RNA SAMPLE, RNA-POLYMERASE PROMOTER, CLEAVAGE FRAGMENT ASSOCIATED GENE IDENTIFICATION, DATABASE, COMPUTER BIOINFORMATIC SOFTWARE BIOINFORMATICS

(21, 49)

=> poly-dT and VN####

POLY-DT IS NOT A RECOGNIZED COMMAND

The previous command name entered was not recognized by the system.  
For a list of commands available to you in the current file, enter  
"HELP COMMANDS" at an arrow prompt (>).

=> spoly-dT and VN####

SPOLY-DT IS NOT A RECOGNIZED COMMAND

The previous command name entered was not recognized by the system.  
For a list of commands available to you in the current file, enter  
"HELP COMMANDS" at an arrow prompt (>).

=> s poly-dT and VN####

L13 4 POLY-DT AND VN####

=> d ibib abs 113 1-4

L13 ANSWER 1 OF 4 MEDLINE on STN

ACCESSION NUMBER: 90196009 MEDLINE

DOCUMENT NUMBER: PubMed ID: 2316523

TITLE: Five polymorphic microsatellite **VNTRs** on the  
human X chromosome.

AUTHOR: Luty J A; Guo Z; Willard H F; Ledbetter D H; Ledbetter S;  
Litt M

CORPORATE SOURCE: Department of Biochemistry, Oregon Health Sciences  
University, Portland 97201-3098.

CONTRACT NUMBER: HD20619 (NICHD)  
R01-GM32500 (NIGMS)

SOURCE: American journal of human genetics, (1990 Apr) 46 (4)  
776-83.

Journal code: 0370475. ISSN: 0002-9297.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199004

ENTRY DATE: Entered STN: 19900601

Last Updated on STN: 19900601

Entered Medline: 19900423

AB The human genome contains approximately 50,000 copies of an interspersed repeat with the sequence (dT.dG/dA.dC)n, where n = approximately 10-60. We and others have found that several of these repeats have variable lengths in different individuals, with allelic fragments varying in size by multiples of 2 bp. These "microsatellite" variable number of tandem repeats (**VNTRs**) may be scored by PCR, using unique flanking primers to amplify the repeat-containing regions and resolving the products on DNA sequencing gels. Since few **VNTRs** have been found on the X chromosome, we screened a flow-sorted X chromosome-specific genomic library for microsatellites. Approximately 25% of the phage clones hybridized to a poly (dT-dG).poly(dA-dC) probe.

Of seven X-linked microsatellites present in positive phages, five are polymorphic and three have both eight or more alleles and heterozygosities exceeding 75%. Using PCR to amplify genomic DNAs from hybrid cell panels, we confirmed the X localization of these **VNTRs** and regionally mapped four of them. The fifth **VNTR** was regionally mapped by virtue of its tight linkage to DXS87 in Centre du Polymorphisme Humain families. We conclude that whatever factors limit the occurrence of "classical" **VNTRs** and RFLPs on the X chromosome do not appear to operate in the case of microsatellite **VNTRs**.

L13 ANSWER 2 OF 4 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.

on STN  
ACCESSION NUMBER: 90113454 EMBASE  
DOCUMENT NUMBER: 1990113454  
TITLE: Five polymorphic microsatellite **VNTRs** on the human X chromosome.  
AUTHOR: Luty J.A.; Guo Z.; Willard H.F.; Ledbetter D.H.; Ledbetter S.; Litt M.  
CORPORATE SOURCE: Department of Biochemistry, Oregon Health Sciences, University, Portland, OR 97201-3098, United States  
SOURCE: American Journal of Human Genetics, (1990) 46/4 (776-783).  
ISSN: 0002-9297 CODEN: AJHGAG  
COUNTRY: United States  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 022 Human Genetics  
LANGUAGE: English  
SUMMARY LANGUAGE: English

AB The human genome contains approximately 50,000 copies of an interspersed repeat with the sequence (dT.dG/dA.dC)n, where n = .apprx.10-60. We and others have found that several of these repeats have variable lengths in different individuals, with allelic fragments varying in size by multiples of 2 bp. These 'microsatellite' variable number of tandem repeats (**VNTRs**) may be scored by PCR, using unique flanking primers to amplify the repeat-containing regions and resolving the products on DNA sequencing gels. Since few **VNTRs** have been found on the X chromosome, we screened a flow-sorted X chromosome-specific genomic library for microsatellites. Approximately 25% of the phage clones hybridized to a poly (dT-dG).poly(dA-dC) probe. Of seven X-linked microsatellites present in positive phages, five are polymorphic and three have both eight or more alleles and heterozygosities exceeding 75%. Using PCR to amplify genomic DNAs from hybrid cell panels, we confirmed the X localization of these **VNTRs** and regionally mapped four of them. The fifth **VNTR** was regionally mapped by virtue of its tight linkage to DXS87 in Centre du Polymorphisme Humain families. We conclude that whatever factors limit the occurrence of 'classical' **VNTRs** and RFLPs on the X chromosome do not appear to operate in the case of microsatellite **VNTRs**.

L13 ANSWER 3 OF 4 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN  
ACCESSION NUMBER: 1990:261676 BIOSIS  
DOCUMENT NUMBER: PREV199090003762; BA90:3762  
TITLE: FIVE POLYMORPHIC MICROSATELLITE **VNTRs** ON THE HUMAN X CHROMOSOME.  
AUTHOR(S): LUTY J A [Reprint author]; GUO Z; WILLARD H F; LEDBETTER D H; LEDBETTER S; LITT M  
CORPORATE SOURCE: DEP BIOCHEMISTRY, OREGON HEALTH SCIENCES UNIV, PORTLAND, OREG 97201-3098, USA  
SOURCE: American Journal of Human Genetics, (1990) Vol. 46, No. 4, pp. 776-783.  
CODEN: AJHGAG. ISSN: 0002-9297.  
DOCUMENT TYPE: Article  
FILE SEGMENT: BA  
LANGUAGE: ENGLISH  
ENTRY DATE: Entered STN: 5 Jun 1990  
Last Updated on STN: 5 Jun 1990

AB The human genome contains approximately 50,000 copies of an interspersed repeat with the sequence (dT · dG/dA · dC)n, where n = .apprx. 10-60. We and others have found that several of these repeats have variable lengths in different individuals, with allelic fragments varying in size by multiples of 2 bp. These "microsatellite" variable number of tandem repeats (**VNTRs**) may be scored by PCR, using unique flanking primers to amplify the repeat-containing regions and resolving the products on DNA sequencing gels. Since few **VNTRs** have been found on the X chromosome, we screened a flow-sorted X chromosome-specific genomic library for microsatellites. Approximately

25% of the phage clones hybridized to a **poly (dT-dG)-poly(dA-dC)** probe. Of seven X-linked microsatellites present in positive phages, five are polymorphic and three have both eight or more alleles and heterozygosities exceeding 75%. Using PCR to amplify genomic DNAs from hybrid cell panels, we confirmed the X localization of these **VNTRs** and regionally mapped four of them. The fifth **VNTR** was regionally mapped by virtue of its tight linkage to DXS87 in Centre du Polymorphisme Humain families. We conclude that whatever factors limit the occurrence of "classical" **VNTRs** and RFLPs on the X chromosome do not appear to operate in the case of microsatellite **VNTRs**.

L13 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2005 ACS on STN  
ACCESSION NUMBER: 1990:401430 CAPLUS  
DOCUMENT NUMBER: 113:1430  
TITLE: Five polymorphic microsatellite **VNTRs** on the human X chromosome  
AUTHOR(S): Luty, J. A.; Guo, Z.; Willard, H. F.; Ledbetter, D. H.; Ledbetter, S.; Litt, M.  
CORPORATE SOURCE: Dep. Biochem., Oregon Health Sci. Univ., Portland, OR, USA  
SOURCE: American Journal of Human Genetics (1990), 46(4), 776-83  
CODEN: AJHGAG; ISSN: 0002-9297  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB The human genome contains approx. 50,000 copies of an interspersed repeat with the sequence (dT.dG/dA.dC)<sub>n</sub>, where n = .apprx.10-60. Several of these repeats have variable lengths in different individuals, with allelic fragments varying in size by multiples of 2 bp. These microsatellite variable number of tandem repeats (**VNTRs**) may be scored by PCR, using unique flanking primers to amplify the repeat-containing regions and resolving the products on DNA sequencing gels. Since few **VNTRs** have been found on the X chromosome, a flow-sorted X chromosome-specific genomic library was screened for microsatellites. Approx. 25% of the phage clones hybridized to a **poly (dT-dG).poly(dA-dC)** probe. Of seven X-linked microsatellites present in pos. phages, 5 are polymorphic and 3 have both 8 or more alleles and heterozygosities exceeding 75%. Using PCR to amplify genomic DNAs from hybrid cell panels, the X localization of these **VNTRs** was confirmed and 4 of them were regionally mapped. The fifth **VNTR** was regionally mapped by virtue of its tight linkage to DXS87 in Center du Polymorphisme Humain families. It was concluded that whatever factors limit the occurrence of classical **VNTRs** and RFLPs on the X chromosome they do not appear to operate in the case of microsatellite **VNTRs**.

=> d his

(FILE 'HOME' ENTERED AT 13:51:22 ON 22 MAR 2005)

FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS'  
ENTERED AT 13:51:31 ON 22 MAR 2005

L1 13 S OLIGO-DT AND VN##  
L2 7 DUP REM L1 (6 DUPLICATES REMOVED)  
L3 20 S DIFFERENTIAL DISPLAY AND VN##  
L4 8 DUP REM L3 (12 DUPLICATES REMOVED)  
L5 5 S VNNN  
L6 2 S PRIMER AND (VNNN### OR VN1N2###)  
L7 0 S VN1N2#  
L8 2 S IDENTIMER  
L9 19 S VN? AND PERMUTATION  
L10 7 DUP REM L9 (12 DUPLICATES REMOVED)  
L11 185 S VNX

L12            1 S L11 AND OLIGO-DT  
L13            4 S POLY-DT AND VN####

=> s poly-dT and VNNN  
L14            0 POLY-DT AND VNNN

=>  
=> polyadenylation an VN?  
POLYADENYLATION IS NOT A RECOGNIZED COMMAND  
The previous command name entered was not recognized by the system.  
For a list of commands available to you in the current file, enter  
"HELP COMMANDS" at an arrow prompt (>).  
  
=> s polyadenylation an VN?  
L15            0 POLYADENYLATION AN VN?

=> d his

(FILE 'HOME' ENTERED AT 13:51:22 ON 22 MAR 2005)

FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS'  
ENTERED AT 13:51:31 ON 22 MAR 2005

L1            13 S OLIGO-DT AND VN###  
L2            7 DUP REM L1 (6 DUPLICATES REMOVED)  
L3            20 S DIFFERENTIAL DISPLAY AND VN###  
L4            8 DUP REM L3 (12 DUPLICATES REMOVED)  
L5            5 S VNNN  
L6            2 S PRIMER AND (VNNN### OR VN1N2###)  
L7            0 S VN1N2#  
L8            2 S IDENTIMER  
L9            19 S VN? AND PERMUTATION  
L10          7 DUP REM L9 (12 DUPLICATES REMOVED)  
L11          185 S VNX  
L12          1 S L11 AND OLIGO-DT  
L13          4 S POLY-DT AND VN###  
L14          0 S POLY-DT AND VNNN  
L15          0 S POLYADENYLATION AN VN?

=> s l11 and (primer or oligo-dT)  
L16          5 L11 AND (PRIMER OR OLIGO-DT)

=> dup rem l15 1-5 ibib abs  
L15 HAS NO ANSWERS

=> d ibib abs 116 1-5

L16 ANSWER 1 OF 5      MEDLINE on STN  
ACCESSION NUMBER: 2003545638      MEDLINE  
DOCUMENT NUMBER: PubMed ID: 14624033  
TITLE: Effects of vomeronasal organ removal on the sperm motility  
in male mice.  
AUTHOR: Koyama Sachiko; Kamimura Shinji  
CORPORATE SOURCE: Division of Biology, Department of Life Sciences, Graduate  
School of Arts & Sciences, University of Tokyo, Meguro-ku,  
Tokyo, Japan.. sakoyama@indiana.edu  
SOURCE: Zoological science, (2003 Nov) 20 (11) 1355-8.  
Journal code: 8702287. ISSN: 0289-0003.  
PUB. COUNTRY: Japan  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200402  
ENTRY DATE: Entered STN: 20031120  
              Last Updated on STN: 20040211

Entered Medline: 20040210

AB Odors play important roles in the communication of house mice. They release behaviors and prime changes of the physiological conditions of other individuals. In our previous study, we showed that sperm motility was lowered in the subordinate mice comparing with dominant mice. Our hypothesis is that the lowered sperm motility was due to some **primer** effects by odor substances derived from dominant mice. To test the hypothesis, we destroyed the vomeronasal organ (VNO) of male mice (**VNX** male) at 5 weeks of age and paired them with intact male mice (Experimental Group). As control group males, intact male mice were kept in pairs (Control Group). At 15 weeks of age, the sperm motility and weights of reproductive organs, and social dominance was analyzed. The subordinate **VNX** males were found to have high sperm motility comparable to the dominant males. It was suggested that there is male-to-male **primer** effects, mediated by VNO, that suppress sperm motility of the subordinate mice.

L16 ANSWER 2 OF 5 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN  
ACCESSION NUMBER: 2002-17928 BIOTECHDS

TITLE: Identifying and characterizing gene expression in samples, for identifying mRNAs expressed at different levels, comprises employing an identimer having a **oligo-dT primer** of a specific sequence and a detectable marker at its 5' end; gene expression identification and characterization, database and computer bioinformatic software

AUTHOR: KANE M D; DOMBKOWSKI A A; NAGEL A C

PATENT ASSIGNEE: GENOMIC SOLUTIONS INC

PATENT INFO: WO 2002036828 10 May 2002

APPLICATION INFO: WO 2000-US45401 1 Nov 2000

PRIORITY INFO: US 2000-244933 1 Nov 2000

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2002-508123 [54]

AN 2002-17928 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - Systems for identification and characterization of gene expression in one or more samples, comprise an identimer having a specific **oligo-dT primer** sequence, where the identimer comprises a detectable marker at its 5' end.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a system (M1) for identification and characterization of gene expression in one or more samples, comprising: (a) providing one or more samples comprising one or more mRNA molecules; (b) providing an identimer comprising an **oligo-dT primer** of sequence, from 5' to 3' end, of (I) or (II), where the identimer also comprises a detectable marker at its 5' end; (c) contacting the mRNA with the identimer so that the polyT portion of the identimer hybridizes to the polyA tail of the mRNA and the **VNx** portion of the identimer hybridizes with portions of the mRNA immediately upstream of the polyA tail; (d) reverse transcribing the mRNA to produce a first strand cDNA that includes the identimer; (e) synthesizing a second DNA strand complementary to the first strand cDNA to form a duplex; (f) cleaving the duplex with a sequence-specific cleaving agent to provide one or more duplex cleavage fragments; (g) ligating an adapter comprising an RNA polymerase promoter site to one or more of the cleavage fragments; and (h) amplifying one or more ligated cleavage fragments using the identimer to produce one or more amplified fragments comprising sequences complementary to a 3' end of the mRNA; (2) a system for identification and characterization of gene expression in one or more samples, by: (a) employing steps (a) - (c) of M1; (b) providing a second sample comprising one or more mRNA molecules; (c) providing an identimer comprising (I) or (II); and (d) employing steps (c)-(h) of M1; (3) a system for identification and characterization of gene expression in one or more

samples, by employing the steps of M1, and further contacting the in vitro transcribed RNA with the identimer so that the polyT portion of the identimer hybridizes to the polyA tail of the in vitro transcribed RNA and the (I) or (II) portion of the identimer hybridizes with portions of the in vitro transcribed RNA immediately upstream of the polyA tail, and reverse transcribing the in vitro transcribed RNA to produce a first strand cDNA that includes the identimer; (4) a system for identification and characterization of gene expression in two or more samples, comprising: (a) employing steps (a) - (c) of M1; (b) providing a second sample comprising one or more mRNA molecules; (c) providing an identimer comprising (I) or (II); (d) employing steps (d) - (h) of M1; (e) contacting the in vitro transcribed RNA with the identimer so that the polyT portion of the identimer hybridizes to the polyA tail of the in vitro transcribed RNA and the (I) or (II) portion of the identimer hybridizes with portions of the in vitro transcribed RNA immediately upstream of the polyA tail; and (f) reverse transcribing the in vitro transcribed RNA to produce a first strand cDNA that includes the identimer; (5) a kit comprising: (a) one or more identimers comprising an **oligo-dT primer** of sequence, from 5' to 3' end, of (I), where the identimer also comprises a detectable marker at its 5' end; and (b) one or more sequence-specific cleaving agents. Tn-**VNx** (I) Tn-VNNN (II) n = an integer 8 or greater but not more than 50 representing the number of T's; V = a nucleotide a, c, or g but not t; N = a nucleotide a, c, g, or t; and x = an integer 3 or greater but not more than 10 representing the number of N nucleotides.

**BIOTECHNOLOGY - Preferred Method:** The system further comprises identifying and characterizing the cleavage fragments according to the presence of the marker, the sequences corresponding to the (I) or (II) nucleotide sequence and the sequence associated with the sequence-specific cleaving agent, and the size of the fragment. The system also includes identifying any gene associated with the cleavage fragments by comparing the sequence and size characteristics of the cleavage fragment with a database contacting sequence and size characteristics of RNAs associated with known genes, where the comparison is conducted by means of software operated on a microprocessor.

**USE -** The system is useful for identifying any or all genes expressed in a given in vivo or in vitro RNA sample, as well as the relative differences in mRNA between 2 or more samples, where desired, for supporting discovery of new genes, and for identifying mRNAs that are expressed at different levels between 2 or more samples.

**ADVANTAGE -** The new system or method addresses limitations of prior methods by comprising compositions and systems that incorporate new strategies where molecular or biochemical assay compositions and systems are linked to DNA or RNA sequence databases for optimal resource efficiency in assaying gene expression. The system has the following advantages over prior and existing methods: (a) prior sequence information or clone library construction is not needed to enable the assay; (b) provides immediate sequence information in addition to information concerning changes or differences in mRNA level, to determine mRNA expression level and mRNA identification in one assay; (c) generates cDNA fragments from all mRNAs present in the sample for subsequent investigation by common molecular biology techniques; and (d) does not require prior knowledge of the sequence of the genome of the organism under investigation and can be employed in organisms lacking significant genomic sequence information.

**EXAMPLE -** Experimental protocols are described but no results were given. (45 pages)

L16 ANSWER 3 OF 5 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN  
ACCESSION NUMBER: 2004:111479 BIOSIS  
DOCUMENT NUMBER: PREV200400112568  
TITLE: Effects of vomeronasal organ removal on the sperm motility in male mice.  
AUTHOR(S): Koyama, Sachiko [Reprint Author]; Kamimura, Shinji

CORPORATE SOURCE: Department of Chemistry, Institute of Pheromone Research,  
Indiana University, 800E Kirkwood Ave., Bloomington, IN,  
47401, USA

sakoyama@indiana.edu

SOURCE: Zoological Science (Tokyo), (November 2003) Vol. 20, No.  
11, pp. 1355-1358. print.

CODEN: ZOSCEX. ISSN: 0289-0003.

DOCUMENT TYPE: Article

LANGUAGE: English

ENTRY DATE: Entered STN: 25 Feb 2004

Last Updated on STN: 25 Feb 2004

AB Odors play important roles in the communication of house mice. They release behaviors and prime changes of the physiological conditions of other individuals. In our previous study, we showed that sperm motility was lowered in the subordinate mice comparing with dominant mice. Our hypothesis is that the lowered sperm motility was due to some **primer** effects by odor substances derived from dominant mice. To test the hypothesis, we destroyed the vomeronasal organ (VNO) of male mice (**VNX** male) at 5 weeks of age and paired them with intact male mice (Experimental Group). As control group males, intact male mice were kept in pairs (Control Group). At 15 weeks of age, the sperm motility and weights of reproductive organs, and social dominance was analyzed. The subordinate **VNX** males were found to have high sperm motility comparable to the dominant males. It was suggested that there is male-to-male **primer** effects, mediated by VNO, that suppress sperm motility of the subordinate mice.

L16 ANSWER 4 OF 5 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on  
STN

ACCESSION NUMBER: 2004:66783 SCISEARCH

THE GENUINE ARTICLE: 759TA

TITLE: Effects of vomeronasal organ removal on the sperm motility in male mice

AUTHOR: Koyama S (Reprint); Kamimura S

CORPORATE SOURCE: Indiana Univ, Dept Chem, Inst Pheromone Res, 800E Kirkwood Ave, Bloomington, IN 47401 USA (Reprint); Univ Tokyo, Grad sch Arts & Sci, Dept Life Sci, Div Biol, Meguro Ku, Tokyo 1538902, Japan

COUNTRY OF AUTHOR: USA; Japan

SOURCE: ZOOLOGICAL SCIENCE, (NOV 2003) Vol. 20, No. 11, pp. 1355-1358.

Publisher: ZOOLOGICAL SOC JAPAN, TOSHIN-BUILDING, HONGO 2-27-2, BUNKYO-KU, TOKYO, 113, JAPAN.

ISSN: 0289-0003.

DOCUMENT TYPE: Article; Journal

LANGUAGE: English

REFERENCE COUNT: 15

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Odors play important roles in the communication of house mice. They release behaviors and prime changes of the physiological conditions of other individuals. In our previous study, we showed that sperm motility was lowered in the subordinate mice comparing with dominant mice. Our hypothesis is that the lowered sperm motility was due to some **primer** effects by odor substances derived from dominant mice. To test the hypothesis, we destroyed the vomeronasal organ (VNO) of male mice (**VNX** male) at 5 weeks of age and paired them with intact male mice (Experimental Group). As control group males, intact male mice were kept in pairs (Control Group). At 15 weeks of age, the sperm motility and weights of reproductive organs, and social dominance was analyzed. The subordinate **VNX** males were found to have high sperm motility comparable to the dominant males. It was suggested that there is male-to-male **primer** effects, mediated by VNO, that suppress sperm motility of the subordinate mice.

L16 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2005 ACS on STN  
 ACCESSION NUMBER: 2002:353661 CAPLUS  
 DOCUMENT NUMBER: 136:351378  
 TITLE: Eukaryotic gene expression detection by reverse transcription with oligo-T primers and database searches  
 INVENTOR(S): Kane, Michael David; Dombkowski, Alan A.; Nagel, Aaron C.  
 PATENT ASSIGNEE(S): Genomic Solutions Inc., USA  
 SOURCE: PCT Int. Appl., 45 pp.  
 CODEN: PIXXD2  
 DOCUMENT TYPE: Patent  
 LANGUAGE: English  
 FAMILY ACC. NUM. COUNT: 1  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002036828	A2	20020510	WO 2001-US45401	20011101
WO 2002036828	A3	20030227		
			W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM	
			RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG	
AU 2002018000	A5	20020515	AU 2002-18000	20011101
US 2003108874	A1	20030612	US 2001-2536	20011101
PRIORITY APPLN. INFO.:			US 2000-244933P	P 20001101
			WO 2001-US45401	W 20011101

AB The invention comprises compns. and systems to identify and compare expressed genes in a given in vivo or in vitro RNA sample, as well as the relative difference in mRNA expression between two or more sample, where desired. Furthermore, the invention comprises compns. and systems to identify novel genes. The invention comprises, without limitation, one or more mRNA specific identimers for use in reverse transcription that themselves comprise an oligo-T nucleotide sequence (at the 5' end) linked to a nucleotide sequence **VNx** (at the 3' end) where the V nucleotide immediately adjacent to the oligo-T segment is not a T. The present invention addresses limitations in the prior art by comprising compns. and systems that incorporate novel strategies whereby mol. or biochem. assay compns. and systems are linked to DNA or RNA sequence databases for optimal resource efficiency in assaying gene expression. Figure 1 shows a mol. protocol of one embodiment of the invention to generate 3' cDNA fragments for the assay of all polyadenylated mRNAs in eukaryotic samples. The sample under investigation is divided into 192 aliquots, and first strand synthesis (reverse transcription) is carried out using all VNNN combinations of the identimer, followed by second strand synthesis. The ds cDNA is cleaved in a sequence-specific manner using a restriction enzyme that involves a 4-base recognition sequence (e.g., Nla III). The resulting fragments are ligated to an adaptamer that contains one or more RNA polymerase promoter sites for subsequent in vitro transcription. The 3' cDNA fragments are initially enriched using PCR, primed at the adaptamer and the polyadenylation site (i.e. identimer), and subsequently employed as a template for in vitro transcription promoted within the adaptamer (e.g. T7 polymerase promoter in the ligated adaptamer). This results in an amplification of the sequence adjacent to, and downstream from, the RNA polymerase promoter sequence, which includes the restriction site and the polyadenylation site. "Second round" first strand synthesis is carried out using a fluorescence-labeled primer (identimer) to enable the detection of all 3' cDNA

fragments for size and abundance (fluorescence label is denoted as an "\*" at the 5' end of the identimer in Figure 1). The entire process is repeated using a different restriction enzyme that employs a different recognition sequence (e.g. MboI). Gene (mRNA) identification is made by collecting knowledge of the 4 nucleotides upstream of the polyadenylation site (determined by identimer priming), the sequence of the restriction enzyme recognition site, and the size of the fragment that provides the distance between the polyadenylation site and the proximal restriction site. This information is employed to search the known sequence database(s) to identify the mRNA(s) that match these criteria.

=> end

ALL L# QUERIES AND ANSWER SETS ARE DELETED AT LOGOFF

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ENTRY	SESSION

FULL ESTIMATED COST

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DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)

SINCE FILE	TOTAL
ENTRY	SESSION

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